OLFACTORY COMMUNICATION

Chemical characterization of the interdigital secretion of the black wildebeest, *Connochaetes gnou*

by

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any other university for a degree.

SUMMARY

The black wildebeest, *Connochaetes gnou*, is a terriorial animal and although it is not generally accepted, it is believed that it defines its territory by scent marking, using interdigital and preorbital secretions, faeces, and urine. The aim of this study was to characterize the chemical constituents of the interdigital secretion. Due to the complexity of the secretion, only one hundred and ten of the approximately 350 compounds could be determined with known techniques. Gas chromatography, low resolution GC-MS and retention-time comparison were the main analytical techniques used. Classes of compounds identified in the interdigital secretion include the following:

- Hydrocarbons – Aliphatic (saturated and unsaturated) and aromatic
- Alcohols – Aliphatic (saturated, unsaturated, cyclic and diols)
- Phenols and Phenylalkanols
- Aldehydes – Aliphatic (saturated and unsaturated) and aromatic
- Ketones – Aliphatic (saturated, unsaturated, cyclic and diketones) and aromatic
- Hydroxy ketones – Aliphatic and cyclic
- Carboxylic acids – Aliphatic (saturated, unsaturated and cyclic) and aromatic
- An anhydride
- Esters – Methyl esters, ethyl and higher esters, unsaturated esters and aromatic esters
- Lactams
- A steroid

Only small qualitative and quantitative differences were found between the male and female interdigital secretions.

OPSOMMING

Die swartwildebees, *Connochaetes gnou*, is 'n territoriale dier en alhoewel dit nie algemeen aanvaar word nie, word vermoed dat hierdie bokke hul gebied afbaken met behulp van interdigitale en preorbitale afskeidings, en deur faeces en urine. Die doel van hierdie studie was om die chemiese samestelling van die interdigitale afskeiding te karakteriseer. As gevolg van die kompleksiteit van die afskeiding, kon slegs eenhonderd-en-tien van die ongeveer 350 verbindings met bekende bestaande tegnieke geïdentifiseer word. Gaschromatografie, lae resolusie GC-MS en retensietyd-vergelyking was die belangrikste analitiese tegnieke wat gebruik is. Klasse van verbindings wat bepaal is, sluit die volgende in:

- Koolwaterstowwe – Alifaties (versadig en onversadig) en aromaties
- Alkohole – Alifaties (versadig, onversadig, siklies en diole)
- Fenole en Fenielalkanole
- Aldehiede – Alifaties (versadig en onversadig) en aromaties
- Ketone – Alifaties (versadig, onversadig, siklies en diketone) en aromaties
- Hidroksiketone – Alifaties en siklies
- Karboksielsure – Alifaties (versadig, onversadig en siklies) en aromaties
- 'n Anhidried
- Esters – Metiel esters, etiel en hoër esters, onversadigde esters en aromatiese esters
- Laktame
- 'n Steroïed

Slegs klein kwalitatiewe en kwantitatiewe verskille is gevind tussen die bul en koei interdigitale afskeidings.

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This famous town of Mansoul has five gates in at which to come, out at which to go; and these were made likewise answerable to the walls, to wit, impregnable, and such as could never be opened nor forced but by the will and leave of those within. The names of the gates were these: Ear-gate, Eye-gate, Mouth-gate, Nose-gate, and Feel-gate.

Bunyan

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CHAPTER 1
INTRODUCTION AND OBJECTIVES

1.1 General introduction

One of the inescapable consequences of man's evolution was that as he raised himself into an upright posture, his nose, once close to the earth, came to be held high above it[1]. Vision and hearing became more acute and gradually the newly emergent human being came to rely less and less upon his nose for information about his environment. As his social organisation developed, ritual became associated largely with visual and acoustic stimulation, although incense is used to this day in a symbolic manner to cleanse the air. Self-produced odours gradually became socially unacceptable and, in a manner not unlike the measures taken to cover the body in many societies, efforts were made to hide them. Freud wrote remarkably little about smell, but what he did say was that odorous sensations connected with the earth became repellent when man rose to his hind feet. The primitive odorous secretions were, he argues, primarily associated with sexual behaviour, so they had to be denied when man started living in social groups more complex than the single family[2]. Historical anecdotes and anthropological reports suggest that man has associated odours with reproductive processes of his own species since the dawn of history[3]. Birth, the attainment of puberty, menstruation, coitus, and pregnancy have direct olfactory associations that provide a rich folklore for the rituals of a number of societies. In a southwest Pacific society studied by Davenport[4], a form of love magic is based upon the similarity of vaginal odours to those of fish. Men attract fish by using a red ground cherry attached to the leader of a trolling line. After having caught a fish, the ground cherry is believed to have the power to attract women. The Trobriand Islanders believe that magic must enter the nose to achieve maximal potency[5]. Thus, charms of love are made from mint and aromatic herbs, which are subsequently placed in bracelets. A number of early medical books state that odours of human urine and other "foul smelling" substances can produce abortion[6], and German folk medicine prescribes rosemary and myrtle to be worn on the breast or in a wreath to protect against pregnancy[7]. The odours of

sweat have been reported to release sexual passions and desires, particularly among the nobility[8].

Today, western man finds himself in a somewhat ambivalent position, for while he will deny that he gains much information about his fellow beings from perceiving their odours, he does nevertheless go to extraordinary lengths not to only remove naturally produced odours, but to replace them with fragrances which agreeably titillate the nasal membranes. There can be little doubt that man's anthropocentric disdain of his olfactory prowess has held up the development of a clear understanding about how the sense of smell works and how it is used. Although progress is now being made quickly in these areas, there is far to go before we have as good an understanding of olfaction as we have of vision and hearing. Worldwide interest in **mammalian olfaction** has been awakened by the fortuitous overlapping of interest in **mammalian behaviour** and in the **analysis of natural secretions**[1]. In our quest for the mechanisms of sensory arousal, we must not lose sight of the need to view the olfactory system as part of an integrated perceptual organ, the systems of which accommodate to ever-changing levels of stimulation, yet allow the animal to make a constant perception about its environment[9].

All but few of the world's 3940 or so species of mammals have a keenly developed sense of smell. *The majority depend for **communication** more on olfaction than vision or hearing and most are richly endowed with scent-producing organs and glands.* Many have developed special behaviour patterns for odour dissemination[10]. The senses of animals are designed for the maintenance of close contact between the organism and the whole of its environment, both biotic and abiotic. *The primary role of olfaction is to make the detection of food and predators possible – its use in **communication** is, in evolutionary terms, a secondary development although it is of fundamental importance in the creation and maintenance of social organisation of mammals and in the control of many facets of behaviour.* In mammals, the olfactory sense is extremely sensitive and appears to be highly developed in almost all terrestrial orders. This widespread occurrence is probably due to the very fast rate of mammalian evolution from primitive stock. Early mammals were small predators which doubtless relied heavily on their noses for detection of their prey. To them, an efficient olfactory system had a strong selective

advantage. Their invasion of almost every possible ecological niche has meant that we now see mammals with highly developed olfactory systems in every part of the world and in almost all habitats[11].

1.2 The pheromone concept in mammalian chemical communication

Studies on mammalian chemical communication have increased dramatically since 1959 when the term "**pheromone**" was first proposed. This surge of research has been due to many interacting factors, including:

- Increased interest in animal behaviour and ecology.
- Advances in micro-organic analytical techniques.
- Successful characterization of natural insect social attractants and repellents.
- Societal needs for non-toxic controls of animal pests.
- Widespread usage of the word "pheromone" in both the scientific and the popular press[12].

Pheromones were initially defined for insects as "substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction, for example, a definite behaviour or a developmental process"[13]. Wright gives another definition of a pheromone: A pheromone is defined as a chemical released by one individual which produces a response in another individual of the same species[14]. Inherent in the original definition was the distinction between "releasing" and "priming" pheromones. **Primer pheromones** affect the physiology of the receiver as manifested by such things as the Bruce Effect (an olfactory pregnancy block in newly mated female mice who fail to conceive if exposed to the odour of alien males), or the remarkable physical and behavioural changes in desert locusts when they pass from solitary to the migratory phase as a result of becoming crowded together. At the human level, perhaps the most striking example of a primer pheromone being at work is McClintock's report of menstrual synchronisation in pairs of women living together in a college dormitory[15]. **Releaser pheromones** release a behavioural response from a physical or

physiological situation. Possible traces of their operation have been reported in such things as the fact that women are more able than men to detect certain male chemical emanations such as boar taint in pork, or the differential effects on men and women of exposure to sex-related odours prior to undertaking some kind of psychological test.

The word "pheromone" is derived from the Greek *pherein* (to carry) and *horman* (to excite, stimulate)[16]. Kirschenblatt has attacked the term pheromone as being etymologically incorrect, as it gives no clue to its exact derivation[17]. Kirschenblatt previously proposed the term "telergones", from the Greek *tele* (afar) and *ergon* (action), to designate all biologically active substances secreted by animals into their environment which influence other organisms[18,19]. Micklem cited the same objection to the term "pheromone" and suggested that it be changed to "pherormone"[20]. In replying to Micklem, Karlson and Lüscher gave a new etymological explanation for their term, stating that the ending "mone" is regarded as a proper suffix used in such scientific terms as "hormones", "gamones" and "termones"[13]. "Pheromone" is now commonly used and widely accepted to include those substances secreted by an animal to influence the behaviour of other animals of the same species[21]. Brown *et al.* have proposed the term "**allomone**" to include those chemical substances produced by an organism which evoke a behavioural or physiological reaction in an organism of another species, and the term "**kairomone**" as a trans-specific chemical messenger of benefit to the recipient rather than to the producer[22]. The term **semiochemical**, derived from the Greek *semeion* (a signal or mark) and *chemeceia* (alchemic), was proposed by Law and Regnier as a general term for all chemicals carrying messages between organisms[23,24] (Fig. 1.1). The distinction between "releaser effect" and "primer effect", introduced by Wilson and Bossert[25], is illustrated in Fig. 1.2. Although the plasticity and variability of mammalian behaviour makes it considerably different from the behaviour of most insects, the majority of authors considering mammals have adhered closely, at least in their theorising, to the original insect definition of a pheromone. Bronson has suggested that "releasing pheromone" be modified to "signalling pheromone" for mammals, since the former implies a degree of innateness which may not characterize many mammalian systems[26,27,28]. The criteria commonly assumed for mammalian pheromones are:

- Species specificity.
- A well-defined behavioural or endocrinological function.
- Any experiment, which definitely isolates a pheromone, should provide evidence of the uniqueness of the isolated compound or small set of compounds in producing the behavioural or endocrinological response[29].

1.3 What is an odour?

An odour is the sensory stimulation from a group of molecules[11]. Only certain molecules possess the necessary properties to enable them to be odorous. The stimulation affects the olfactory membrane of the nose and associated structures in a way that is not yet fully understood. Normally the molecules are encountered as a stream, or plume, emanating from the producer organ. Unlike acoustic or visual signals which travel very fast from producer to receiver, odours travel rather slowly. In still air they diffuse according to Graham's laws of gas diffusion. When helped by air movement, however, odours may travel remarkably fast. From a point source, the concentration at various distances from that source is given by the expression

$$U(r,t) = \frac{Q}{2D\pi^{r}} f\left[\frac{r}{\sqrt{4Dt}}\right]$$

where the concentration $U(r,t)$ is measured in molecules/cm^3; r is the distance (cm) from the emission; t is the time (seconds) from the start of emission; Q is the emission rate of the gas (molecules/s); D is the diffusion coefficient of the substance in air (cm^2/s) and f is a complex correction function for error. This model is important since it enables an investigator to estimate K, the threshold concentration, which is the value of $U(r,t)$ at which a behavioural or physiological response in the animal can be detected and recorded[30] (Table 1.1).

Table 1.1: Odour thresholds (K) for humans of some pure compounds

Compound	Threshold [μg per litre of water]
Acetone	450 000
Ethanol	100 000
Butyric acid	250
Butanal	70
Amyl acetate	5
Dimethyl sulphide	0.3
Methanethiol	0.02
β-ionone	0.007
2-methoxy-3-isobutylpyrazine	0.002

A notable advantage of odorous over visual or acoustic communication is that the signal can continue to be sent out long after the animal producing it has moved away. Wilson and Bossert calculated that 1 gram of gyplure ($C_{18}H_{34}O_3$), the sex attractant of the gypsy moth, would require over ten million years to fade out to below the threshold level if it were released altogether in one puff in perfectly still air[25].

1.4 The olfactory system of vertebrates

Titus Lucretius Carus was probably the first person to try to understand why different substances have different odours. In 49 BC he wrote that it was impossible to suppose that sweet smelling substances had atoms of the same shape as foul smelling substances. He inferred that the former were round and smooth while the latter were rough and hooked[31].

The universality of olfaction in the vertebrates is reflected in the anatomical simplicity of its system. In stark contrast to the anatomical position of visual and acoustic receptor cells, which are guarded by membranes, fluid baths, bones and other structures serving to transmit and attenuate the signal waves before they are perceived, olfactory receptor cells boldly protrude into the environment, unshielded save for a thin ever-changing veil of mucus. The first structure

encountered by an inspired odorant molecule is an exposed sensory cell that is no less than a forward projection of the olfactory centre of the brain. The structure of the gustatory, or taste, system is very similar and equally universal in its occurrence. The olfactory system is triggered when a stream of molecules from a source enters the nasal cavities and makes contact with the **sensory epithelium** or sensory membrane. The olfactory receptors are every bit as sensitive to change in the quality of the incoming signals as are the visual and acoustic receptors. Thus a complex stream of messages can be received and analysed. Each sensory system possessed by vertebrates is specialised to be maximally effective under a restricted, and different, set of environmental conditions. For the perception of an odour, it is necessary only for the perceiver to be close enough to the odour source so that some scented molecules can be swept up into its nose. The overwhelming advantage of olfactory signalling over visual and acoustic signalling is that the signaller need not be present at the site of odour emanation – all that is required is the presence of some molecules which could have been left behind by the signaller at some earlier time. As far as food finding is concerned, this is a distinct disadvantage, unless the perceptive system is able to cope with a concentration gradient caused by a temporally induced decay, but it is highly advantageous in most social situations.

The anatomy of the olfactory apparatus is remarkably similar throughout the class Vertebrata. There is an inlet to, and an outlet from, a chamber in which is held a thin sensory membrane of sometimes immense area. In the terrestrial vertebrates the outlet from the chamber enters the respiratory tract, posterior to the soft palate. The olfactory epithelium of vertebrates has a structure which shows remarkably little variation between classes. It consists of a basal membrane lying on the turbinal bone which supports three main cell types (Fig. 1.3):

- Receptor cells.
- Ciliated support cells.
- Basal cells.

In addition there are mucus-secreting Bowman's glands. The whole membrane is about 95 μm thick. The receptor cells are bipolar structures which terminate in a swelling known as the olfactory knob. Arising from the knob are a series of

filamentous cilia, the actual number of which appears to be species-specific. Since the cilia are the first parts of the nervous system encountered by an incoming odorant molecule, it would seem reasonable to expect that specific adsorption sites might be present on their peripheral membranes. After the odorant has triggered the adsorption site, the impulses travel along the cilium towards the knob, but a single triggering may influence more than just one cell. At intervals along the cilium are swellings or vesicles which may coalesce with others from other cilia. Furthermore, the very tip of the taper carries a vesicle which frequently can be seen to be closely appressed to the terminal vesicle of another cilium. Associated with the vertebrate olfactory epithelium is a pigment which is black in fishes, amphibia and reptiles, and brown to brownish-yellow in mammals. In fish and amphibia there are distinct pigment cells lying in the epithelium, but in mammals the pigment seems to be associated with the support cells and Bowman's glands. By far the majority of vertebrates have paired external nares through which air, or water, is drawn for odour detection. The exceptions to this are the lampreys and hagfish, which have a single naris formed from fusion of the basic pair, and several species of birds which lack external nares altogether.

Before considering the structure of the neural pathways leading to the brain, it is necessary to examine first the accessory olfactory organs. These are structures which are associated with the main olfactory system, but have a separate and distinct innervation. Of these, the most obvious is **the organ of Jacobson**, sometimes called the vomeronasal organ (Fig. 1.4). Recent anatomical studies using electron-microscopy techniques have indicated the existence of three other accessory olfactory organs in mammals. These are **the septal organ of Rudolfo-Masera**; **the terminal endings of the *nervus terminalis***; and **the terminal endings of the *nervus trigeminus***. The olfactory bulbs in primitive vertebrates lie anterior to the brain, connected to it *via* the olfactory nerve. In higher vertebrates, they come to lie underneath the brain, overlain by the cerebral hemispheres. The olfactory bulbs function as a special "odour brain" to process the signals before they reach the brain proper[32]. The axons of the receptor cells terminate in the glomeruli, discrete clusters of neuroterminals, which, when viewed all together, constitute the *stratum*

glomerulare[33]. Summary of the five neural components by which mammals detect odour:

- The true olfactory neuro-epithelium.
- The organ of Jacobson.
- The septal organ of Rudolfo-Masera.
- The terminal endings of the *nervus terminalis*.
- The terminal endings of the *nervus trigeminus*.

1.5 Odour-producing organs of mammals

Vertebrates show a wide range of scent deposition behaviours, each one designed to place the odorous secretion in that part of the environment where it will be most noticed by conspecifics. Most specialised behaviours are derived from behaviour associated with elimination, i.e., urination and defecation, though the application of scent secretions from some specialised sebaceous glands requires the acquisition of new behaviour patterns. In general, it appears that active scent setting is a mammalian phenomenon; in snakes and lizards scent which is produced by the dorsal and cloacal glands disperses passively as the animals travel through the vegetation[34]. The production of behaviourally significant odours by mammals occurs in most organs which pass chemicals to the external environment. These organs may have been specifically modified for this purpose, or may liberate odoriferous chemicals as a result of their prime function. The major sources of such odours are the **integument**, the **salivary glands**, the **accessory glands** of the **eye**, **urine** and **vaginal secretions**.

1.5.1 The integument

Odour production by the integument is generally confined to the skin glands. The epidermis may add some components to these glandular secretions, but the role of the epidermis in this respect would seem to be negligible when compared to the volume of secretions produced by skin glands. There are two types of skin gland, **sebaceous glands** and **sweat glands** (Fig. 1.5). Sebaceous glands have a

holocrine secretory process, and pass their secretion *via* a duct into the pilosebaceous canal. Sweat glands may be divided into **apocrine** and **eccrine** glands. Apocrine glands develop from the side of the hair follicle, whereas eccrine glands develop from ectoderm which is not associated with hair follicles. A phylogenetic relationship exists between apocrine and eccrine sweat glands, in that most mammals possess apocrine glands over their entire body surface, whereas higher primates have both apocrine and eccrine glands, with eccrine glands being most numerous in man. Other mammals may have eccrine sweat glands in restricted regions, for example where the skin is differentiated to resist frictional stress, such as the volar surface of paws and digits[35].

1.5.2 Salivary glands

Salivary glands develop from the ectoderm of the oral mucosa. They have a complex branching duct system which terminates in secretory acini. Acini are roughly spherical structures composed of pyramid-shaped secretory cells arranged around a central lumen. These secretory cells rest upon a basement membrane, and elongated or star-shaped myoepithelial cells are present between the basement membrane and the secretory cells. Acini are classified as serous, mucous or mixed according to the nature of their secretory cells, and occur in varying proportions in any one salivary gland.

1.5.3 Accessory glands of the eye

The Harderian gland is a tubulo-alveolar gland, with alveoli composed of cells which contain lipid droplets[36]. There are two types of alveolar cell, Type I and Type II; both are columnar but the Type I cell is small with few lipid droplets and the Type II cell is larger, more numerous, and contains many lipid droplets. The lumen of these alveoli may contain porphyrins[37]. The secretions produced by these cells are passed into the orbit along ducts lined by a pseudo-stratified epithelium[36].

1.5.4 Urine

The pheromonal properties of urine have been described for many species, for example rodents[28], deer[38] and primates[39]. Such urinary odours may in fact originate from accessory sexual organs such as the preputial glands[40]. They also may arise by mixing urine with secretions from accessory sexual organs such as the coagulating glands[41], but also may be present in bladder urine[42].

1.5.5 Vaginal secretions

Vaginal secretions have pheromonal properties in many species[43]. The production of these pheromones seems to depend upon the bacteria of the vagina, and the ovarian hormones which exert their influence on odour production in the intact animal, by determining the availability of nutrients for the bacteria. The nature of these nutrients appears to be epithelial cells and mucus within the vagina[44,45].

1.6 Insect chemical communication

Insects have managed to persist in hostile surroundings because they have developed extraordinary adaptations or abilities, one of which is a highly specialised sense of smell. Because many insects depend on their sense of smell for survival, they can frequently be attracted to a trap by a chemical for detection purposes, to a toxicant that destroys them, or to a substance that makes them incapable of fertile mating[46]. As long ago as 1837, von Siebold recognised that a pair of appendages, sometimes coloured, opening into the vagina of the females of some insect species may act as an attractant for males. He surmised that the odour emitted by a female insect probably functions to entice the male, while that emitted by a male may be used as a stimulus in copulation (aphrodisiac)[47]. The following are only a few examples of insect communication that have been reported.

1.6.1 Acarina

Amblyomma americanum (L.), lone star tick

Amblyomma maculatum Koch, Gulf Coast tick

Dermacentor variabilis (Say), American dog tick

Females of these insects produce a pheromone that attracts males of the respective species. Males respond only after reaching a state of maturity initiated by feeding[48].

Mantis religiosa (L.), praying mantis

Caged virgin females can lure large numbers of males from a distance of up to 100 m between 8:30 am and 1:00 pm[49].

1.6.2 Hemiptera

Lygus hesperus Knight, lygus bug

Virgin females in field traps attracted males. Mating reduces the attractiveness of females for only a few days[50].

1.6.3 Trichoptera

Sericostoma personatum (Spence)

A strong odour of vanilla emitted by scent glands on the maxillary palpi of males is thought to be attractive or excitatory to females[51].

1.6.4 Diptera

Ceratitis capitata (Wiedemann), Mediterranean fruit fly

Mature virgin females are attracted over a short distance and sexually excited by a volatile chemical substance emanating from the erectile anal ampuls of sexually mature males[52].

1.7 Reported identification of mammalian pheromones

The following are a few examples of the identification of mammalian pheromones.

1.7.1 Male pronghorn subauricular pheromone

In 1974 Müller-Schwarze *et al.* reported the identification of the active compound in the subauricular scent of male pronghorns (*Antilocapra americana*). While the social significance of the deer's marking with subauricular glands is not completely understood, responses elicited by marks, including sniffing, licking, marking, and thrashing, were also elicited to an equal degree by a single compound isolated from the mark, namely 3-methylbutanoic acid[53].

1.7.2 Pheromonal secretion from gerbil sebaceous glands

The identification of another mammalian pheromone was reported by Thiessen *et al.* in 1974. A single compound, phenylacetic acid, obtained from ventral sebaceous glands of male Mongolian gerbils (*Meriones unguiculatus*) elicited investigation from other male gerbils. In the initial bioassay, the gerbils were trained to suppress bar pressing for food pellets in the presence of whole sebum odours. Using this procedure, phenylacetic acid inhibited bar pressing to a similar extent as whole sebum odours. In the critical second bioassay procedure, where "exploratory interest" in the whole sebum as well as in various fractions were tested, males investigated the sebum and phenylacetic acid for a longer period than the other fractions[54].

1.7.3 Boar odours stimulating sexual receptivity in the sow

Melrose *et al.* report that two steroids (5-androst-16-en-3-one and its related alcohol) found in the submaxillary salivary gland of boars are responsible for their characteristically tainted breath. Both the breath of boars and an aerosol of either

the alcohol or the ketone increases the incidence of positive reactions to the "back pressure" test in sows. However, the same behavioural response can also be elicited by a mixture of boars' preputial fluid and urine or by warmed preputial secretion[55]. Neither the preputial gland nor urine from boars contain the two steroids found in the salivary glands, illustrating how a stereotyped component of sexual behaviour can be elicited by more than one chemical[56].

1.8 Antelope

The word "antelope" means "bright-eyed" and is generally used, very loosely, to denote the small group of gazelles and their relative the springbuck – all slender, graceful, medium-sized animals. However, the term antelope is used here to cover all species which belong to the Bovid family, having the following characteristic features:

- An even number of toes (artiodactyls).
- They all have hooves (ungulates).
- They chew their cud and have four-chambered stomachs (ruminants).
- All the males have simple, unbranched, bone-filled horns which are never shed (carnicorniates); the females of some species also have horns.
- The upper incisor teeth are absent.

The majority of Bovids occur in Africa (85 species); there are no true Bovids in the Americas except for the pronghorned antelope and the bison, while there are only a few in Asia such as the nilghai, blackbuck and waterbuffalo. The classification of the Bovids represented in Southern Africa is as follows:

Class: MAMMALIA (milk producing animals)

Order: ARTIODACTYLA (even-toed ungulates)

Family: BOVIDAE (ox-like)

The sub-families are:

- BOVINAE:

  Tribe Bovini: 1 species - African buffalo.

  Tribe Tragelaphini: 5 species – eland, kudu, nyala, bushbuck, sitatunga. (Twisted, unringed horns; face and body patterned with spots and stripes.)

- CEPHALOPHINAE:

  3 species – grey, red and blue duikers. (Small, squat, narrow-faced antelope with spike-like horns; facial scent glands and head tufts.)

- ALCELAPHINAE:

  6 species – blue wildebeest, **black wildebeest**, red hartebeest, Lichtenstein's hartebeest, tsessebe. The blesbok and bontebok are variations of one species. (Large, long-faced antelope with high shoulders and sloping backs.)

- ANTELOPINAE:

  Tribe Antelopini: 1 species – springbuck.

  Tribe Neotragine: 7 species – oribi, steenbok, klipspringer, Cape and Sharpe's grysbok, dik-dik and suni. (Small animals with spike-like horns; the females are hornless.)

- AEPYCEROTINAE:

  The impala.

- PELEINAE:

  The vaal rhebuck: This animal, like the impala, has been isolated and placed in its own sub-family. It is considered to be a primitive form, closely allied to the goats.

- HIPPOTRAGINAE:

  (Latin for horse-goats.)

  3 species – gemsbok, sable and roan antelopes.

- REDUNCINAE:

  5 species – waterbuck, puku, red lechwe, southern reedbuck and mountain reedbuck. (Only the males carry horns, which are ringed and curved strongly forward.)

The anatomical design of antelopes shows a very high degree of adaptation to their herbivorous way of life and to the need to escape from predators. The first antelope were small and hornless and their fossil record goes back 65 million years. They have extremely sharp vision with a very wide angle of view and their hearing and **sense of smell** are superb. Their limbs are long, slender and supple and the axis of the foot passes between the second and third toes, which have hooves: the other

toes are rudimentary or have disappeared. This design enables them to balance and to run over any terrain with great speed – many are capable of prodigious leaps, up to 10 meters long, giving them an excellent means of escape. The digestive system is also highly specialised, and utilises bacteria which ferments the high cellulose content of their herbivorous diet and converts it to sugars which provide their main source of energy-giving food. A certain amount of water is also produced as a by-product of cellulose breakdown. The horns, invariably present in the males of all species and also in the females of many species, are generally considered to be used for defence against predators and one another, but it appears that their most important function is their value as display symbols and for ritualistic fighting which is so important in their breeding behaviour. Antelopes have a variety of **scent glands** opening through their skin at various places, according to the species. These have very important functions, which include the recognition of individuals and young, and sexual attraction, herd order and the demarcation of territory which is achieved by rubbing the scent gland onto sticks, grass or the earth at certain points in their territory (Fig. 1.6). Their colouration varies considerably and is thought to be valuable as a form of camouflage.

Antelope are found throughout Africa in almost every conceivable habitat, including the swamps, mountains, forests, deserts and grasslands, due to their ability to exploit a wide range of plant material, including herbs, grasses, fresh and dry leaves, bark, fruits and berries and even underground bulbs which are dug out with their hooves (Fig. 1.7). Many antelope have the ability to do without water for long periods at a time, while some desert species never need to drink, allowing them to exploit waterless places such as the vast plains of the Kalahari. The reproductive cycle in antelopes is typical of most other mammals. Most species breed only once a year, a single "calf" being born at the season of the year which is most advantageous, in most cases at the onset of the rainy season. Under undisturbed conditions, a dynamic balance occurs between predators, the prey species, and the habitat. Predators on antelopes include all the large cats, wild dogs, hyaenas, pythons and large birds of prey[57].

1.9 Black wildebeest (*Connochaetes gnou*)

1.9.1 Description

The shoulder height of the male is about 120 cm and that of the female is about 115 cm. The mass of the male is about 180 kg. They have a peculiar appearance: a powerful body, the back slopes from the massive humped shoulders to the slender, lightly built hindquarters. Black wildebeest have a big head with a conspicuous brush of long hair along the muzzle between the eyes and the nostrils, a distinct chin beard and an elongated patch of hair on the chest that extends to the forelegs. Both sexes carry horns which bend sharply downwards, forwards and upwards (Fig. 1.8).

1.9.2 Colouring

Dark brown, adult bulls can be almost black in colour. Their neck and shoulder manes are yellowish-white at the base but dark towards the tips. The characteristic feature in the field is the tail, which is dark at the base, the remainder with long, off-white hair reaching nearly to the ground. Both sexes have a similar appearance, cows being slightly smaller. Young calves are usually lighter in colour (Fig. 1.9).

1.9.3 Habitat

Black wildebeest prefer the open grassland, sub-desert steppes and dry pans with thornbush savannah.

1.9.4 Distribution

The range of the species in historical times has been limited to the central inland plateau of South Africa, specifically in the Orange Free State, the highveld

regions of the southern, central, and northern Cape Province, the southern Transvaal, and marginally in the grassveld regions of Natal in the foothills of the Drakensberg Range (Fig. 1.10 and Table 1.2).

Table 1.2: Number of black wildebeest in South Africa

Province or Zoo	**1945**	**1965**	**1970**
Orange Free State	754	1216	1935
Cape Province	215	311	508
Transvaal	46	177	475
Natal	17	75	174
Pretoria Zoo	9	6	10
Johannesburg Zoo	6	7	9
Bloemfontein Zoo	1	4	9
Estimated total	**1048**	**1808**	**3120**

1.9.5 Main food

Black wildebeest are predominantly grazers, but they will also feed on succulents and browse on karroid bushes. They often kneel to graze. They depend on water and drink regularly.

1.9.6 Breeding and gestation

They are seasonal breeders; the peak of calving takes place during the summer (December or January) after a gestation period of 240 – 260 days.

1.9.7 Age

Black wildebeest can live up to 20 years.

1.9.8 Voice

Loud roaring snort, sometimes a whistle.

1.9.9 Habits

Black wildebeest are gregarious, occurring in herds of 10 to 30, with several bulls. Solitary bulls are territorially confined to one area, they cannot be driven out of their area very easily. Territorial bulls adopt their characteristic threat display with the neck held erect and the head directed horizontally, as they advance their rivals in a "rocking horse" canter. Forming a half circle, they will come to a standstill and remain in this position until they head off for the next run. The territorial males mark their territories by spreading secretions of the facial and pedal glands on convenient objects and defend them from trespassers by pawing, kneeling and horning the ground, by vocalising with the characteristic loud *ge-nu* and advertising their ownership by cantering around, stiff-legged[58,59,60,61].

1.9.10 Scent marking

Black wildebeest have well-developed interdigital glands on the forelegs and primitive preorbital glands. Pawing with the forelegs is performed by all sexes and age classes and precedes lying down, rolling and defecation, the latter only by territorial bulls. Territorial bulls especially paw vigorously on their stamping grounds, though also away from it. It seems very likely that the sticky secretion from the interdigital cavities is transferred to his stamping ground. If a female herd stays in the territory they quite often also proceed to paw and roll on the bulls's stamping ground. The preorbital glands are shallow and cannot be opened or closed at will. The secretion impregnates the long tufts of hair which cover the glandular area. Males frequently horn the ground or rub their foreheads on the stamping ground. However, the position and the shape of the horns make it highly unlikely that any secretion can be transferred to the ground. A territorial male defecates most frequently on his stamping ground and paws vigorously prior to this; the other members of a wildebeest population, including the bachelor bulls, defecate at

random and without pawing. Defecation also occurs during a challenge ritual. Urination takes place mostly during the challenge ritual and could denote threat. Demarcation by scent (interdigital and preorbital secretions, faeces and urine) was found not to be for territorial purposes. No evidence could be found that another territorial bull took exception to the specific smell of a neighbour. Not infrequently during a challenge ritual both contestants defecate and urinate on one and the same stamping ground. Also females, yearlings and calves will use the stamping ground for defecation and rolling. As the territorial male frequently lies and rolls on his stamping ground, the secretion of his interdigital cavities and faeces will permeate his coat and give him a specific odour of his own. When the bull moves around he is constantly surrounded by it. It is advocated that this gives him self-assurance[62].

1.10 Objectives of this study

The black wildebeest, *Connochaetes gnou*, has several means of demarcating its habitat: interdigital and preorbital glands, faeces and urine. It is not yet clear if this is done for territorial or social purposes, or both. The main objective of this study was firstly to characterize the chemical constituents of the interdigital secretion of the black wildebeest and secondly to comment on the quantitative and qualitative differences between the male and female secretion. It is hoped that this study will not only further the understanding of the territorial and social behaviour of black wildebeest, but also the understanding of the species as a whole.

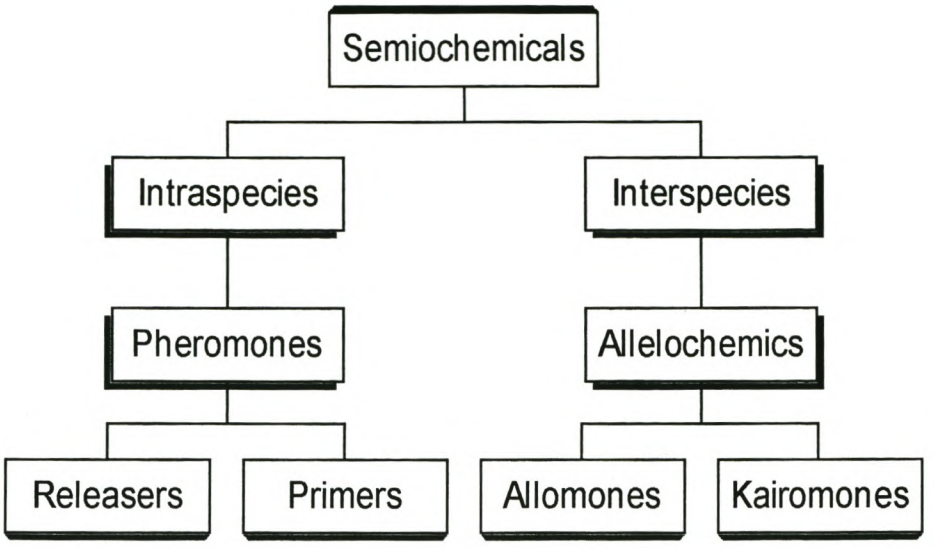

Fig. 1.1: The pheromone concept

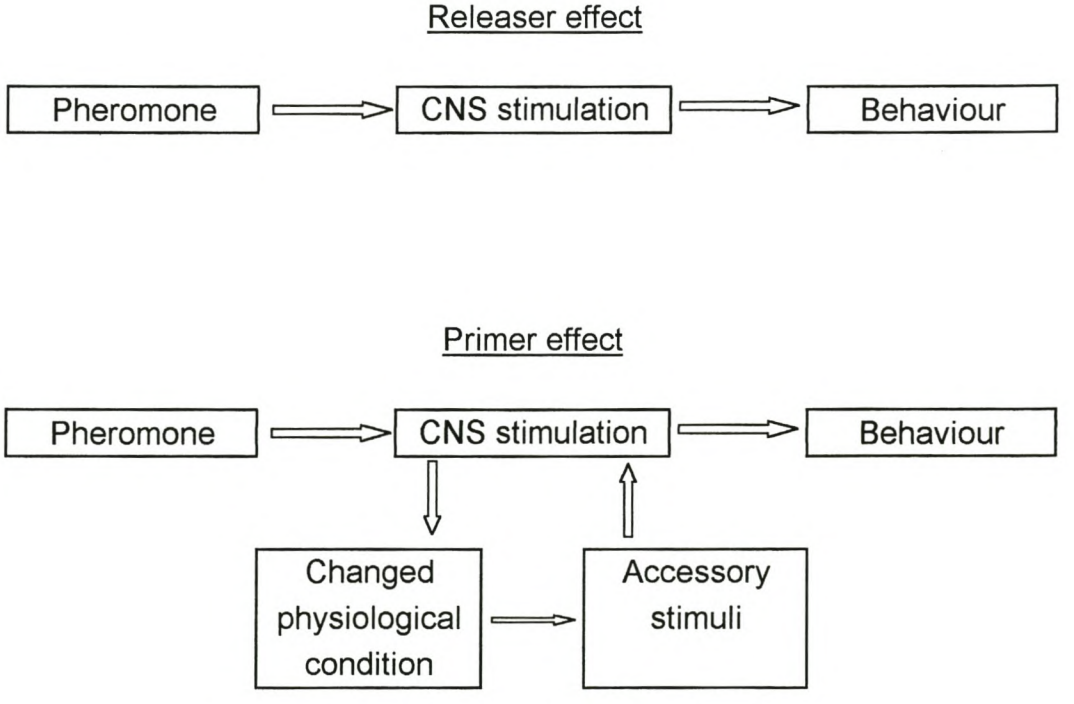

Fig. 1.2: Difference between releaser and primer pheromones

CNS = Central nervous system

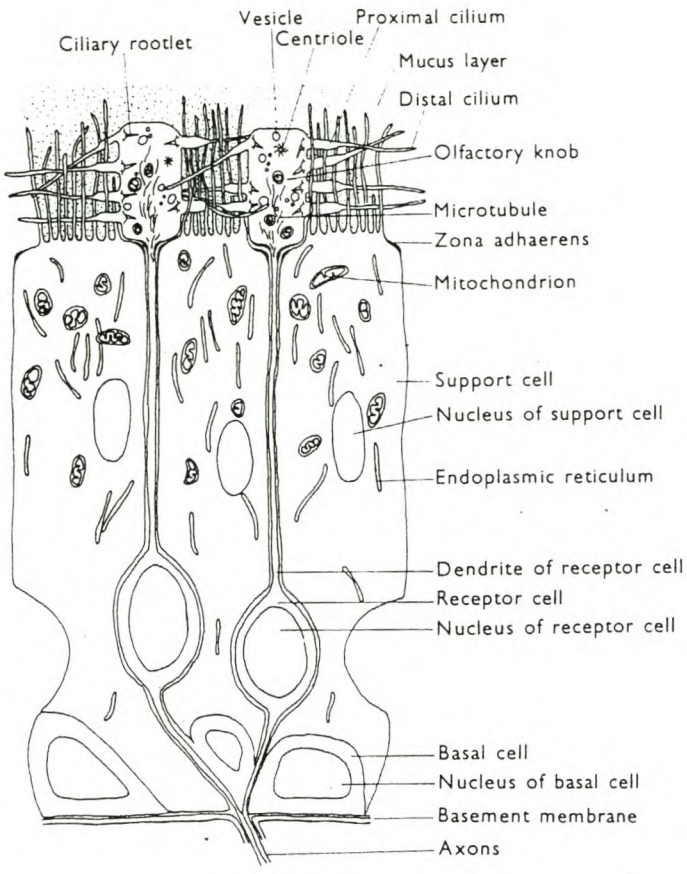

Fig. 1.3: Schematic representation of the components of the olfactory epithelium

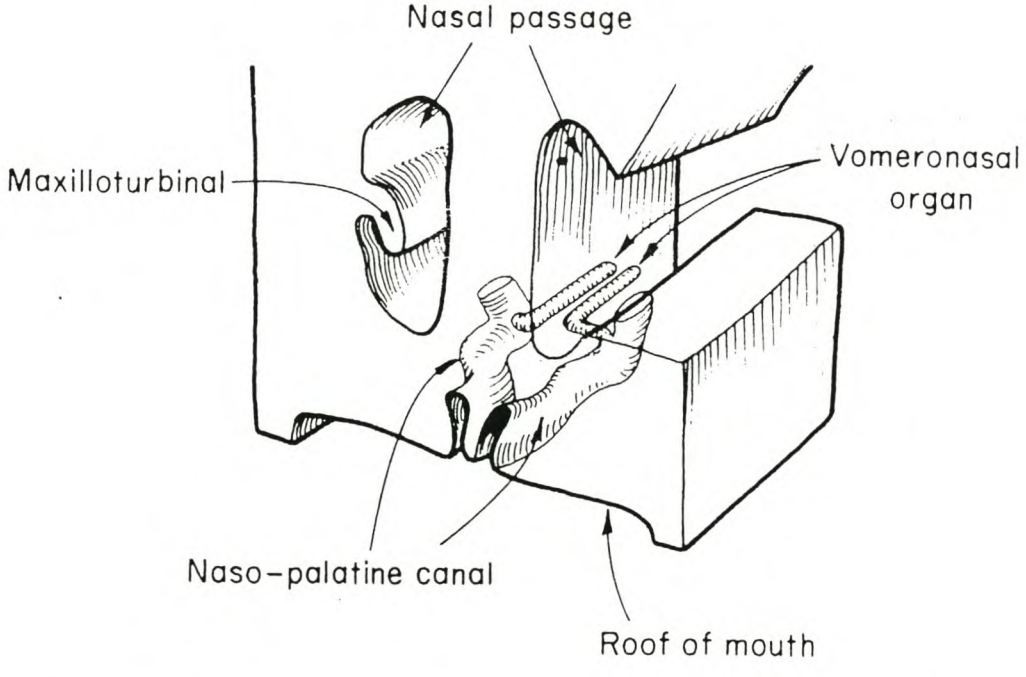

Fig. 1.4: Block diagram of the organ of Jacobson and the nasal passage of the hedgehog

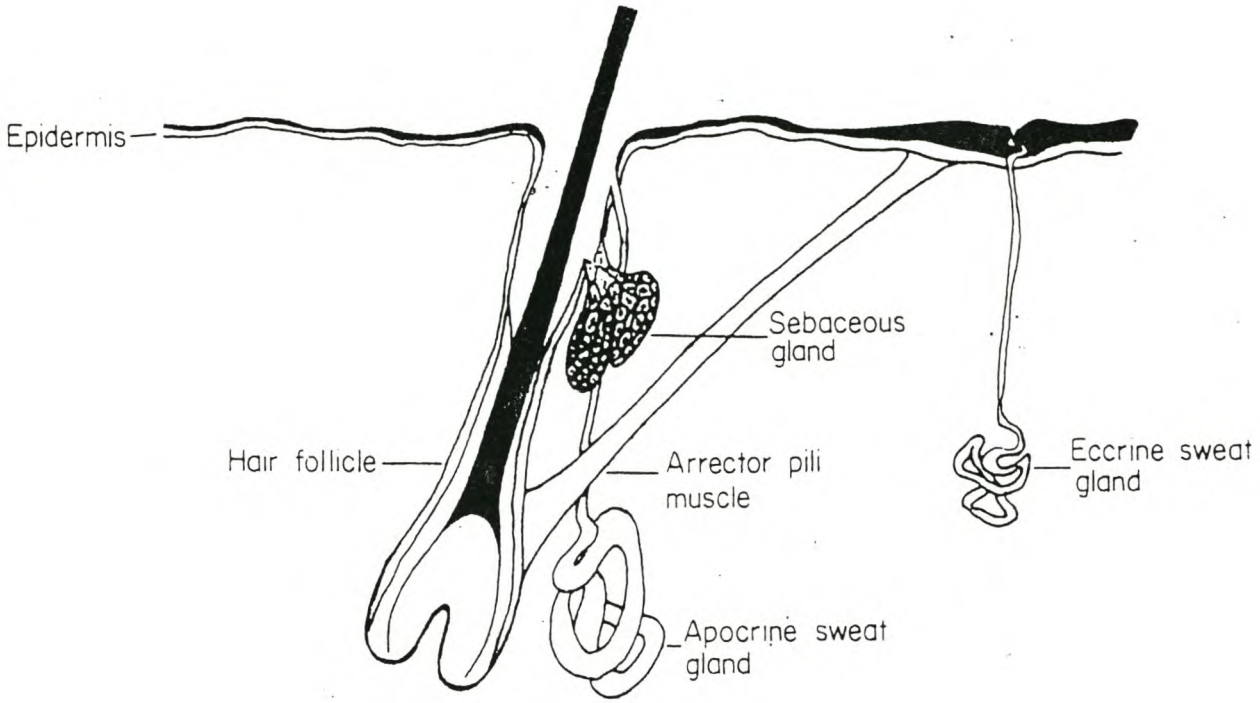

Fig. 1.5: Generalised diagram showing the location of mammalian skin glands

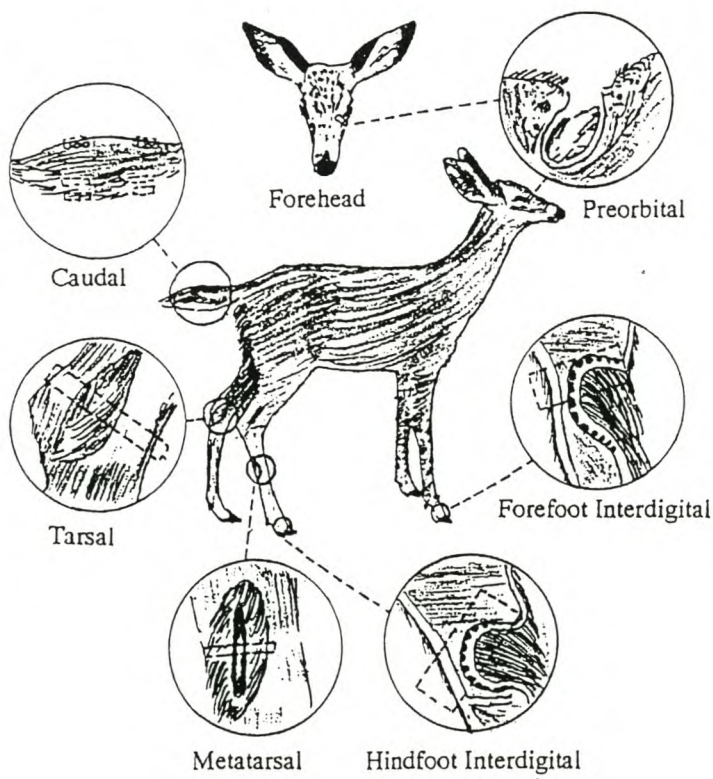

Fig. 1.6: Diagrammed locations of the glandular and integumentary regions

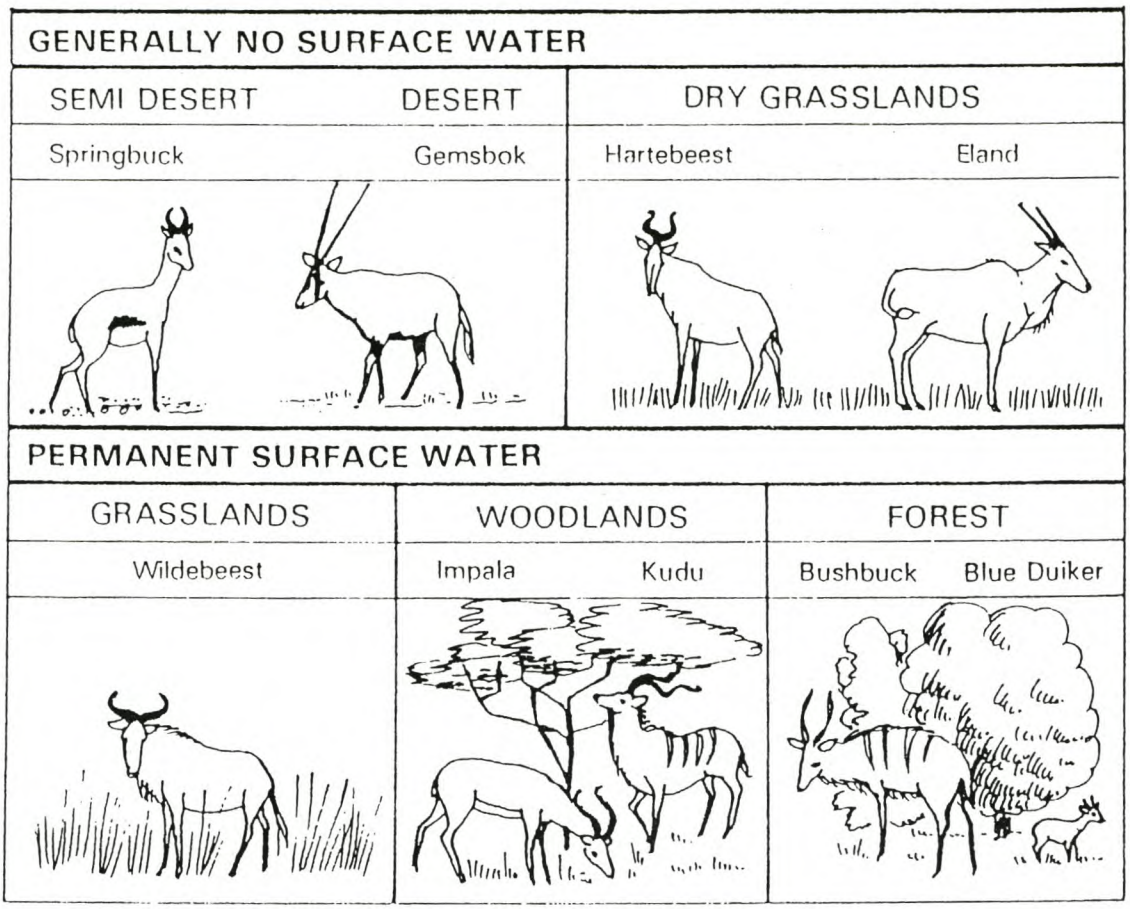

Fig. 1.7: Habitat of antelopes

Fig. 1.8: The horns of the black wildebeest

Fig. 1.9: The black wildebeest, *Connochaetes gnou*

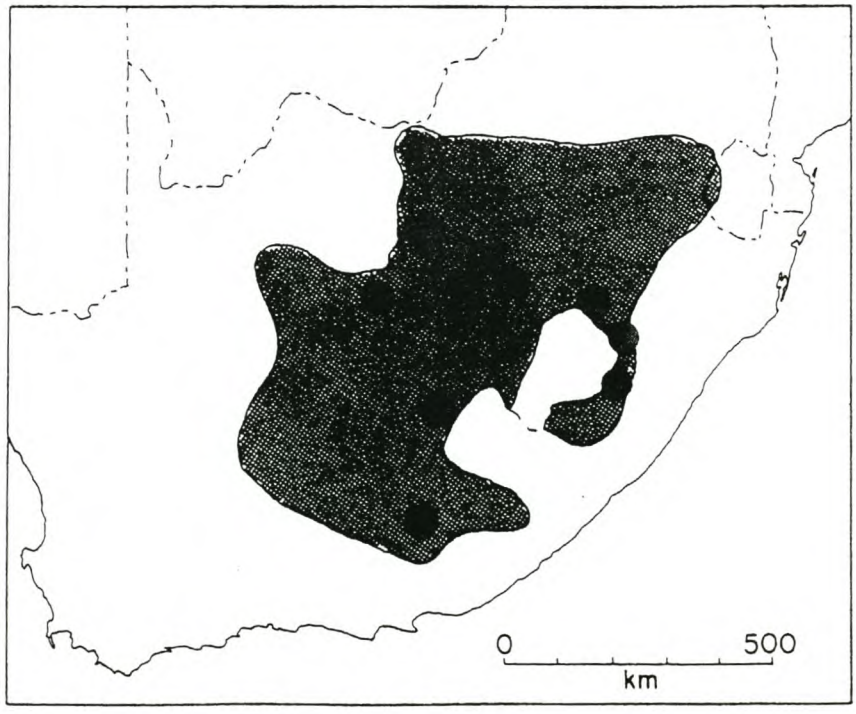

Fig. 1.10: Distribution of the black wildebeest

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CHAPTER 2
CHEMICAL CHARACTERIZATION OF THE INTERDIGITAL SECRETION OF THE BLACK WILDEBEEST, *CONNOCHAETES GNOU*

2.1 Introduction

The total ion chromatograms (TIC) of the male (Fig. 2.1) and female (Fig. 2.2) black wildebeest interdigital secretion will be used as reference in the discussion of the different components in this secretion. Each component will be referred to by its number in these gas chromatograms. Where it was impossible to obtain a pure mass spectrum from either of these gas chromatograms, the mass spectrum from a previous GS-MS analysis was used, so that some of the component reference numbers will not correspond to the scan number shown on the mass spectrum under discussion (Table 2.1).

Individual components were identified using their low resolution electron impact (EI) mass spectra and this was confirmed by gas chromatographic retention time comparison with either authentic, commercially available compounds, or compounds synthesized from authentic material in the laboratory. The term "co-injection", used throughout this study, implies the simultaneous injection of the natural extract and the synthetic reference compound into the gas chromatograph to determine if the reference compound co-elutes with the component under discussion. This, together with comparative mass spectra comparison, is taken as evidence for the compound being present in the natural secretion.

2.2 Structural determination of the components of the interdigital secretion of the black wildebeest

2.2.1 Hydrocarbons: Aliphatic (Saturated)

Saturated hydrocarbons are ionized by the removal of an electron from a σ-bond:

$$R{-}CH_2{-}CH_2{-}R' \xrightarrow{-e^-} R{-}CH_2 \cdot CH_2{-}\overline{R'}\,\big]^+$$

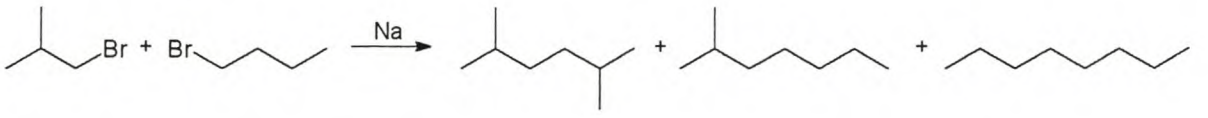
Unbranched hydrocarbons exhibit clusters of ions, 14 atomic mass units (CH_2) apart, of decreasing abundance with increasing fragment mass[1]. The most abundant species in each cluster correspond to the general formulae $[C_nH_{2n+1}]^+$ (m/z 29, 43, 57, 71, ...), and to a lesser extent $[C_nH_{2n-1}]^+$ (m/z 27, 41, 55, 69, ...), with abundance maxima around C3 or C4[2]. The molecular ion is usually present, albeit of low intensity. Branching causes a preferential cleavage at the point of branching, resulting in a decrease in the molecular ion intensity and characteristic increases in the abundance of $[C_nH_{2n+1}]^+$ and $[C_nH_{2n}]^{+\cdot}$. Cleavage and charge retention takes place at the branched carbon, while loss of the largest alkyl group is favoured. This causes a sharp break in the normal pattern of a gradual decline in the abundance of fragments with increasing fragment mass of the unbranched hydrocarbons, which can be used to determine the point of branching. This is due to the better stabilisation of a secondary over a primary carbonium ion. An exception is the loss of an isopropyl group from isoalkanes, with charge retention on the primary ion[3]:

$$RCH_2{-}CH(CH_3)_2 \xrightarrow{-e^-} R\overset{+}{C}H_2 + \dot{C}H(CH_3)_2$$

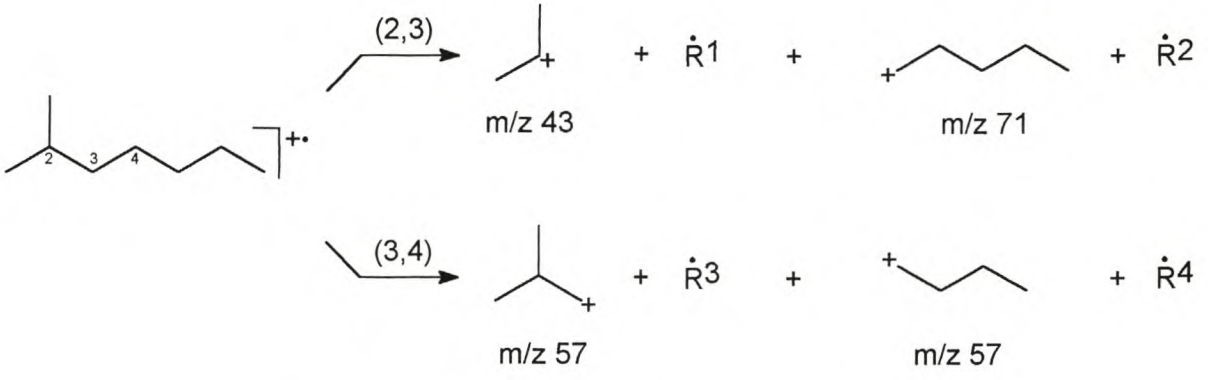
The EI mass spectra of components 412 (Fig. 2.3) and 1070 (Fig. 2.4) in the total ion chromatogram of the interdigital secretion of the black wildebeest (Fig. 2.1) exhibit the mentioned clusters of ions, 14 atomic mass units apart, which are typical of unbranched hydrocarbons. Component 412 has a distinct molecular ion at m/z 114 (C_8H_{18}) and component 1070 has a distinct molecular ion at m/z 142 ($C_{10}H_{22}$), which favours their identification as unbranched alkanes. Co-injection of a series of alkanes (hexane through undecane) with the natural extract, established that components 412 and 1070 are in fact octane and decane, respectively.

The EI mass spectrum of component 343 (Fig. 2.5) also exhibits the mentioned clusters of ions, but with the exception that the C4 ion at m/z 57 is the base peak and no molecular ion is present. As discussed above, this was taken as an indication of branching and more specifically, of an isopropyl group being present in the compound. Since the retention time of this component lies between that of

heptane and octane, component 412, it was assumed that component 343 could be an isooctane, and 2,5-dimethylhexane and 2-methylheptane were synthesized together according to the scheme below [see § 3.4.1 and mass spectra Figs. 3.1(a) and 3.1(b)] and retention-time tested. Co-injection of the synthetic product and the natural extract proved that component 343 is in fact 2-methylheptane.



The formation of the ions at m/z 43, 57 and 71 can be explained as follows:

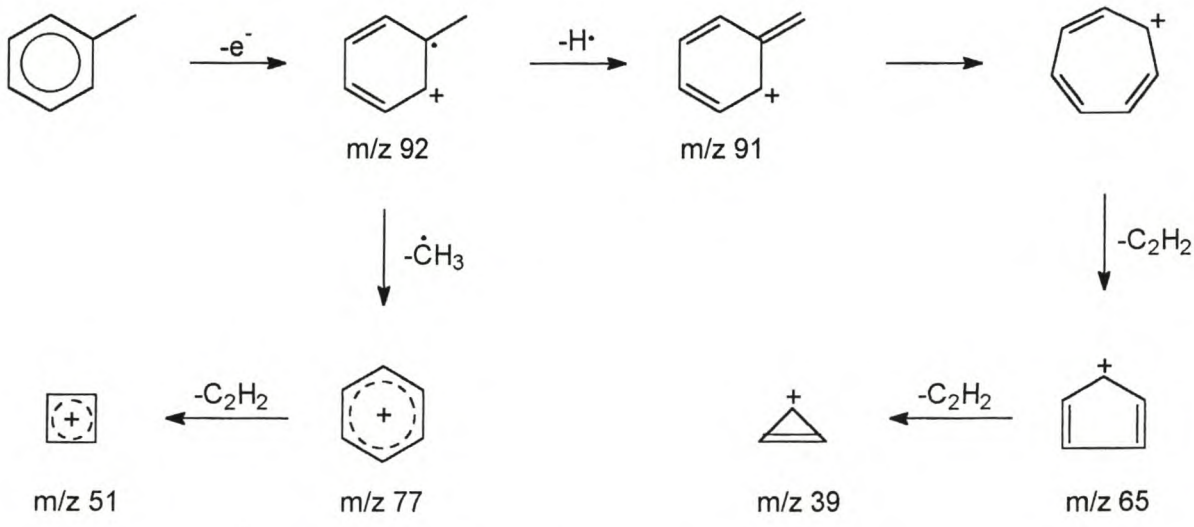


2.2.2 Hydrocarbons: Aliphatic (Unsaturated)

The EI mass spectrum of component 330 (Fig. 2.6) has a base peak at m/z 81 and an ion 14 atomic mass units higher at m/z 95 (10%). Since the loss of 14 atomic mass units is highly unlikely, the ion at m/z 95 cannot be the molecular ion and must therefore, for example, be an $[M-1]^+$ or $[M-15]^+$ ion. Since it is known that acetylenes display a noticeable $[M-1]^+$ ion for 1-pentyne and higher homologues and that ions of the general formula $[C_nH_{2n-3}]^+$ (m/z 39, 53, 67, 81, 95, ...) are of high abundance in acetylenes[4], it was assumed that this component could be 1-heptyne (C_7H_{12}, 96 Da). This was further confirmed in the literature, where it is stated that m/z 81 $[C_6H_9]^+$ is the base peak for 1-heptyne and 1-octyne, formed by the loss of a methyl and an ethyl radical, respectively[4]. Co-injection of commercially available 1-heptyne with the natural extract confirmed that component 330 is 1-heptyne.

2.2.3 Hydrocarbons: Aromatic

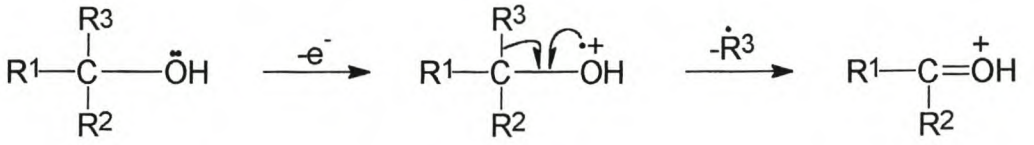
The EI mass spectrum of component 450 (Fig. 2.7) has as its base peak the familiar tropylium ion $[C_7H_7]^+$ at m/z 91 and a molecular ion at m/z 92. This is characteristic for toluene and co-injection of the natural extract with the commercially available product confirmed this. The fragmentation pattern of toluene can be explained as follows[5]:



The EI mass spectra of components 723 (Fig. 2.8) and 751 (Fig. 2.9) are mutually comparable and similar to that of toluene, component 450 (Fig. 2.7), except that they have molecular ions at m/z 106. The base peak at m/z 91 is due to the loss of 15 atomic mass units, which represents a methyl group (CH_3). This means that components 723 and 751 could be homologues of toluene with one of the aromatic hydrogen atoms or one of the alkyl hydrogen atoms substituted by a methyl group; in other words, that components 723 and 751 could be ethylbenzene or one of the xylenes. Co-injection of synthetic ethylbenzene with the natural extract confirmed that component 723 is ethylbenzene. Co-injection of firstly *p*-xylene and then *m*-xylene with the natural extract confirmed that component 751 is *p*-xylene. Due to the higher boiling point of *o*-xylene, and the resulting longer retention time than those of the other two xylenes, this isomer was not considered.

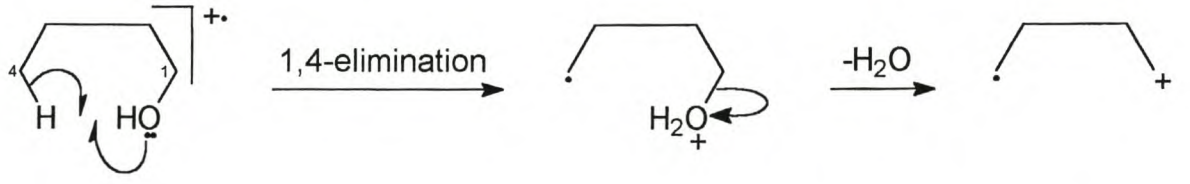
2.2.4 Alcohols: Aliphatic (Saturated)

Components 3553, 3929, 4632 and 4962 in the total ion chromatogram (TIC) of the interdigital secretion of the black wildebeest (Fig. 2.1), have EI mass spectra (Figs. 2.10, 2.11, 2.12 and 2.13, respectively) in which two series of prominent ions appear at m/z 41, 55, 69, *etc.* and m/z 43, 57, 71, *etc.* These ion series correspond to the general formulae $[C_nH_{2n-1}]^+$ and $[C_nH_{2n+1}]^+$, respectively, and show a decreasing relative abundance with increasing fragment mass, typically found in the spectra of long-chain unbranched 1-alkanols[6] and 1-alkenes. The molecular ions of primary and secondary alcohols are usually weak; those of tertiary alcohols are usually not detectable. The most important general fragmentation process involves cleavage of the bond β to the oxygen atom. The largest group is lost most readily[7]:

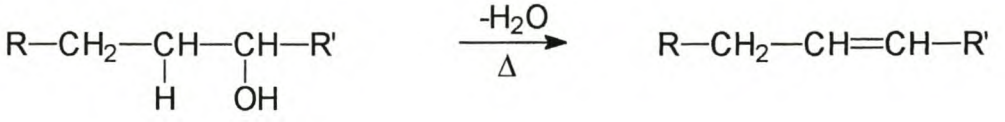


$(R^3 > R^2 > R^1)$

The elimination of water from the molecular ion often results in an $[M-18]^{+\cdot}$ ion. This process proceeds predominantly by 1,4-elimination *via* the following six-membered intermediate[8]:



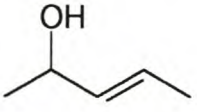
Heated inlet systems in the mass spectrometer or the hot metal surfaces of the ion source can lead to thermal 1,2-elimination of water prior to ionization[7]:



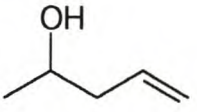
A concurrent elimination of water and ethylene $[M-46]^{+\cdot}$ is usually observed with alcohols of four or more carbon atoms. Depending on the chain length of the compound, such ethylene expulsions can be repeated[9]. If it is assumed that the ions at m/z 140, 154, 182 and 196 in the EI mass spectra of components 3553, 3929, 4632 and 4962 are due to the simultaneous loss of water and ethylene, the molecular mass of each component must be 186, 200, 228 and 242 Da, respectively. As a working hypothesis, it was therefore assumed that component 3553 is 1-dodecanol, component 3929 1-tridecanol, component 4632 1-pentadecanol and component 4962 1-hexadecanol. In the case of these components the $[M-18]^{+\cdot}$ ion, due to the elimination of a water molecule, is not visible in the normalized mass spectrum. By co-injection of a series of synthetic 1-alcohols (1-butanol through 1-eicosanol) with the natural extract, it was confirmed that components 3553, 3929, 4632 and 4962 are 1-dodecanol, 1-tridecanol, 1-pentadecanol and 1-hexadecanol, respectively.

2.2.5 Alcohols: Aliphatic (Unsaturated)

Component 398 (Fig. 2.14) has a molecular ion at m/z 86 (8%) and a base peak at m/z 71 $[M-CH_3]^+$. The EI mass spectrum also has ions at m/z 68 $[M-H_2O]^{+\cdot}$, 53 $[M-CH_3-H_2O]^+$, 45 (11%) and 31 (2%). The m/z 45 ion usually indicates a 2-alkanol and the low relative abundance of this ion together with the molecular ion at m/z 86, were taken as probable indications that component 398 could be penten-2-ol. This information was used in a computer search[13] which gave the following three possibilities:



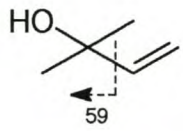
3-Penten-2-ol



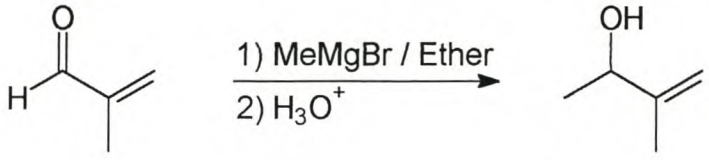
4-Penten-2-ol

2-Methyl-3-buten-2-ol

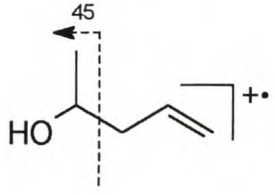
2-Methyl-3-buten-2-ol was discarded because of the presence of an m/z 59 ion in the mass spectrum of this tertiary alcohol, which is not present in the mass spectrum of component 398:



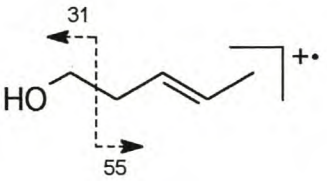
Synthetic 3- and 4-penten-2-ol were tested and it was found that the retention time of the former was too long while that of the latter was too short. The computer search was repeated; this time m/z 71 was not given as the base peak. This gave one additional candidate structure, namely 3-methyl-3-buten-2-ol, which was synthesized according to the scheme shown below (see § 3.4.2 and mass spectrum Fig. 3.2). Co-injection of the synthetic product with the natural extract proved that component 398 is 3-methyl-3-buten-2-ol.



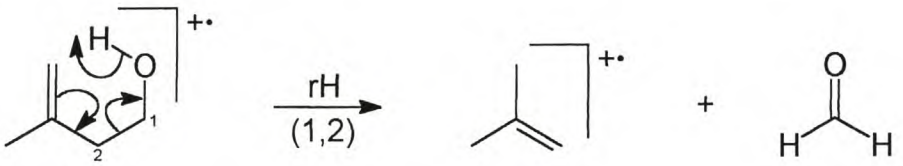
The elimination of 18 atomic mass units (H_2O) from the molecular ion at m/z 86 to give an ion at m/z 68, the presence of an m/z 31 ion and the McLafferty rearrangement ion at m/z 56 in the EI mass spectrum of component 525 (Fig. 2.15), indicated that it could be 3-buten-1-ol with a methyl group in position 2, 3 or 4, giving 2-methyl-3-buten-1-ol, 3-methyl-3-buten-1-ol or 3-penten-1-ol, respectively, as possibilities. Since 4-penten-2-ol has a base peak at m/z 45 owing to the fragmentation shown below and does not give an m/z 56 McLafferty rearrangement ion, it was not considered:



3-Penten-1-ol has a very strong m/z 55 ion, due to α-fission and was therefore also eliminated as a possible candidate:



Synthetic 3-methyl-3-buten-1-ol was tested first and co-injection with the natural extract proved that component 525 is indeed 3-methyl-3-buten-1-ol. The formation of the McLafferty rearrangement ion at m/z 56 can be explained as follows[10]:



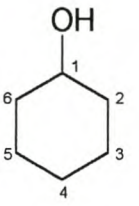
m/z 86 m/z 56

rH = Hydrogen rearrangement

2.2.6 Alcohols: Cyclic

Component 1575 has an EI mass spectrum (Fig. 2.16) with a base peak at m/z 95 and clusters of ions around m/z 41, 57, 69 and 85. The other two ions of interest are present at m/z 110 (22%) and m/z 113 (4%). If these two ions are taken to be the $[M\text{-}H_2O]^{+\cdot}$ and $[M\text{-}CH_3]^+$ ions, respectively, the molecular ion should be at m/z 128 (110 + 18 = 113 + 15 = 128 Da). Closer inspection of the mass spectrum of component 1575 did not confirm this, but as a first hypothesis, it was assumed to be the case. A molecular mass of 128 Da suggests the following oxygen containing molecular formulae as the most likely candidates: $C_8H_{16}O$, $C_7H_{12}O_2$ and $C_6H_8O_3$. Other molecular formulae such as C_6H_8OS and C_6H_5ClO, also giving a molecular mass of 128 Da, were discarded at first because of the biological origin of the material under investigation. The loss of H_2O favours an alcohol and therefore $C_8H_{16}O$ was first considered as a possible molecular formula, which implied that component 1575 could either be an unsaturated or a cyclic alcohol. The m/z 57 ion is a very prominent ion in cyclic alcohols[11] and therefore component 1575 was assumed to be a cyclic alcohol with at least one methyl substituent on the ring, explaining the m/z 113 $[M\text{-}CH_3]^+$ ion. It is known that the elimination of water is

especially noticeable in cyclohexanols[12], and therefore this species was examined first. There are twelve possible structures for dimethylcyclohexanol:

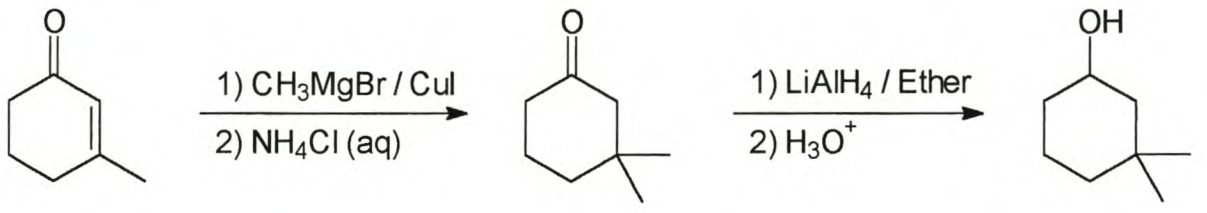


Position of the first methyl group	Position of the second methyl group
1	2, 3, 4
2	2, 3, 4, 5, 6
3	3, 4, 5
4	4

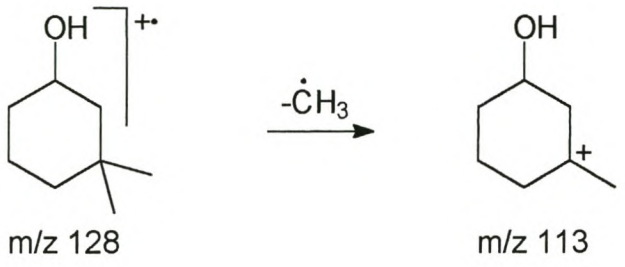
The possibility of an ethyl substituted cyclohexanol was not considered because of the absence of an $[M\text{-}CH_2CH_3]^+$ ion at m/z 99 in the mass spectrum. A computer search using the following parameters was done[13]:

Molecular mass	:	128 Da
Molecular formula	:	$C_8H_{16}O$
Name fragments	:	cyclohexanol & dimethyl

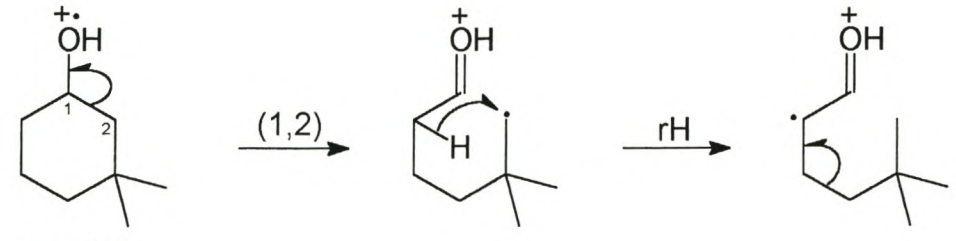
This yielded the following seven compounds as possible candidate structures: 1,2-, 2,3-, 2,5-, 2,6-, 3,3-, 3,4-, and 3,5-dimethylcyclohexanol. If a base peak at m/z 95 is included as a further parameter in the computer search, the results were narrowed down to one possibility, namely 3,3-dimethylcyclohexanol. This compound was synthesized according to the following scheme [see § 3.4.3 and mass spectrum Fig. 3.3(b)]:



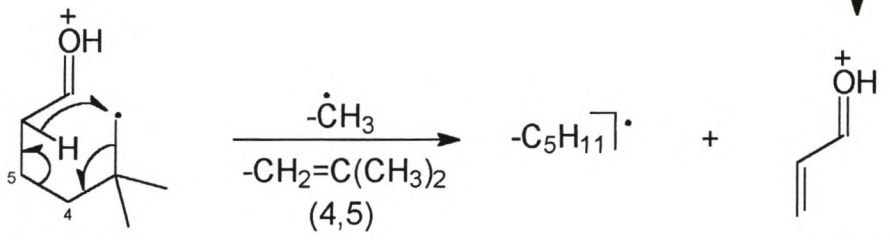
Co-injection of the synthetic product with the natural extract proved that component 1575 is indeed 3,3-dimethylcyclohexanol. The formation of the ions at m/z 113, 110 and 57 (two alternative mechanisms) can be explained as follows[14]:



m/z 128 m/z 113



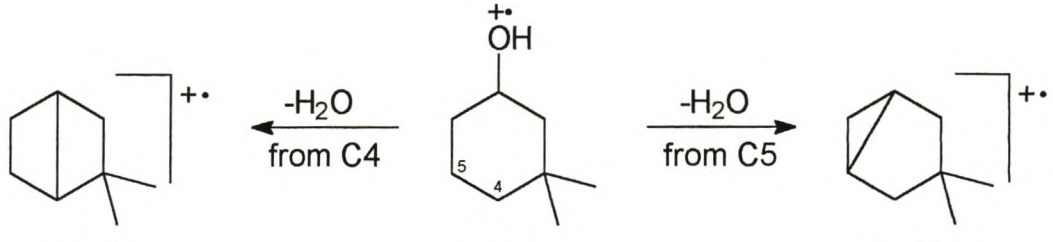
m/z 128
(Mechanism 1)



m/z 128
(Mechanism 2)

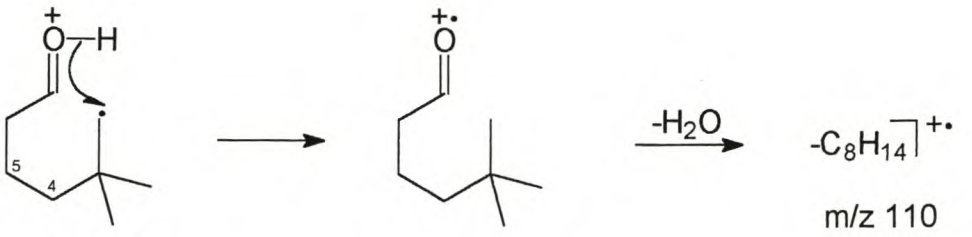
m/z 57

The predominant elimination of water[14] takes place through the loss of the hydroxyl group and a hydrogen atom from either C4 or C5:

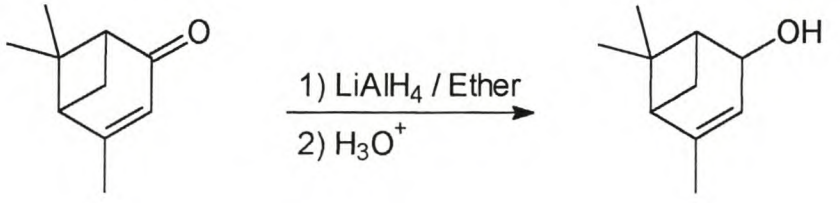


m/z 110 m/z 128 m/z 110

A second dehydration process, occurring to a lesser extent, does not involve the hydrogen atom of the hydroxyl group, but only hydrogen atoms attached to C4 and C5:



The ions at m/z 119, 134 and 137 in the EI mass spectrum of component 2207 (Fig. 2.17) were assumed to be the $[M-H_2O-CH_3]^+$, $[M-H_2O]^{+\cdot}$ and $[M-CH_3]^+$ ions, respectively. This would suggest a molecular mass of 152 Da, corresponding to a molecular formula of $C_{10}H_{16}O$. Using this information together with a relative intensity of 75-100% for the ions at m/z 91 and m/z 109, returned verbenol as a possible candidate[13]. Since verbenone, component 2672 (see § 2.2.15), had already been identified, verbenol was synthesized by the reduction of verbenone [see § 3.4.4 and mass spectrum Fig. 3.4(b)]:

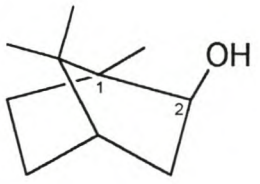


Comparison of the mass spectra of the synthetic product and component 2207 was favorable and co-injection with the natural extract proved that component 2207 is verbenol.

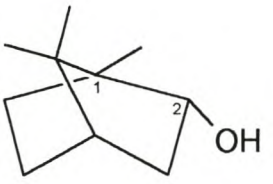
The EI mass spectrum of component 2334 (Fig. 2.18) has a base peak at m/z 95, with all the other ions having an abundance lower than 20%. The ions at m/z 139, 136 and 121 were assumed to be the now familiar $[M-CH_3]^+$, $[M-H_2O]^{+\cdot}$ and $[M-CH_3-H_2O]^+$ ions, respectively, so that component 2334 must have a molecular mass of 154 Da, and a molecular formula containing at least one oxygen atom. A computer search was done using the following parameters[13]:

Molecular mass	:	154 Da
Number of oxygen atoms	:	one or two
Base peak	:	m/z 95
Molecular ion abundance	:	0-5%
$[M-CH_3]^+$ and $[M-H_2O]^{+\cdot}$ abundance	:	2-20%

The search gave two borneol isomers, namely 1-borneol and isoborneol, as possible candidates:



1-Borneol

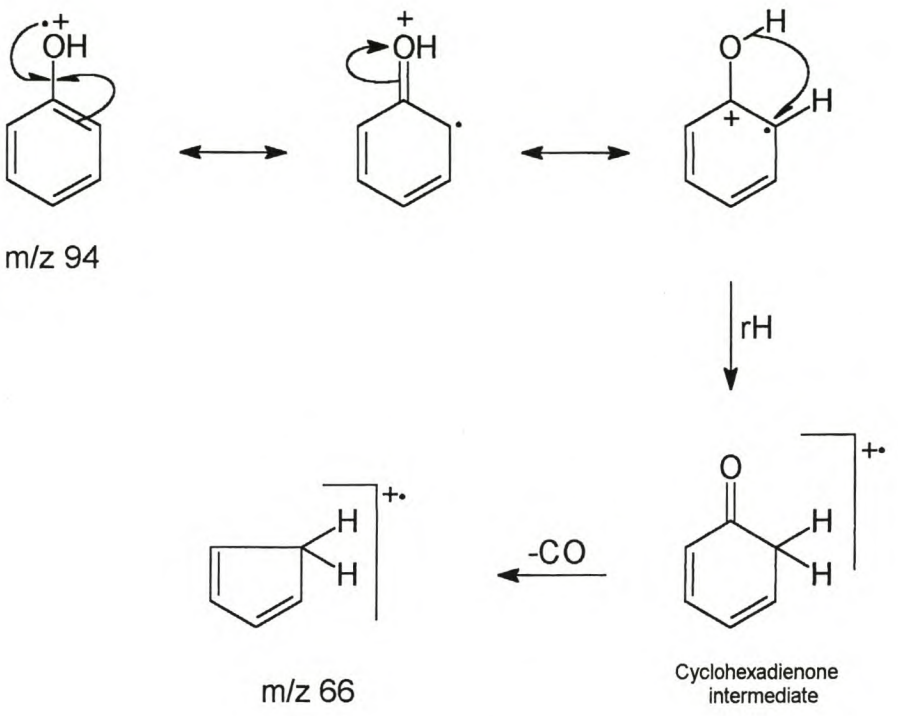


Isoborneol

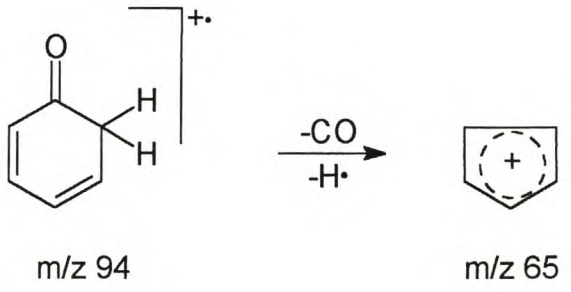
Individual co-injection of these two commercially available compounds with the natural extract proved that component 2334 is 1-borneol.

2.2.7 Phenols and Phenylalkanols

The base peak in the EI mass spectrum of component 2058 (Fig. 2.19) at m/z 94, in addition to the general appearance of the spectrum, suggests that the unknown could be phenol[15], in agreement with the results of a computerized library search[13]. The molecular ion at m/z 94 is accompanied by a weak $[M-1]^+$ ion, which is characteristic of phenol. The most significant fragment in the spectrum, however, is the $[M-28]^+$ ion at m/z 66. Using accurate mass measurements and deuterium labelling, the expulsion of CO has been demonstrated to take place *via* a cyclohexadienone intermediate[16]:



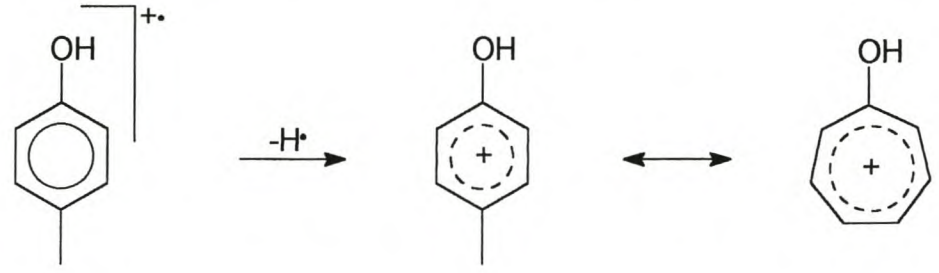
The only other significant ion in the spectrum at m/z 65 is of only slightly reduced abundance and is produced by the loss of the elements CHO from the molecular ion. Deuterium labelling of the phenolic hydrogen atom revealed that only 33% of the hydrogen which is expelled has its origin from this source, the other 67% arising from random extraction from the ring[17]:



Based on the above evidence, it was accepted that component 2058 is phenol, and the identification was confirmed in the usual manner by co-injection of the synthetic compound with the natural material, resulting in co-elution of the two compounds.

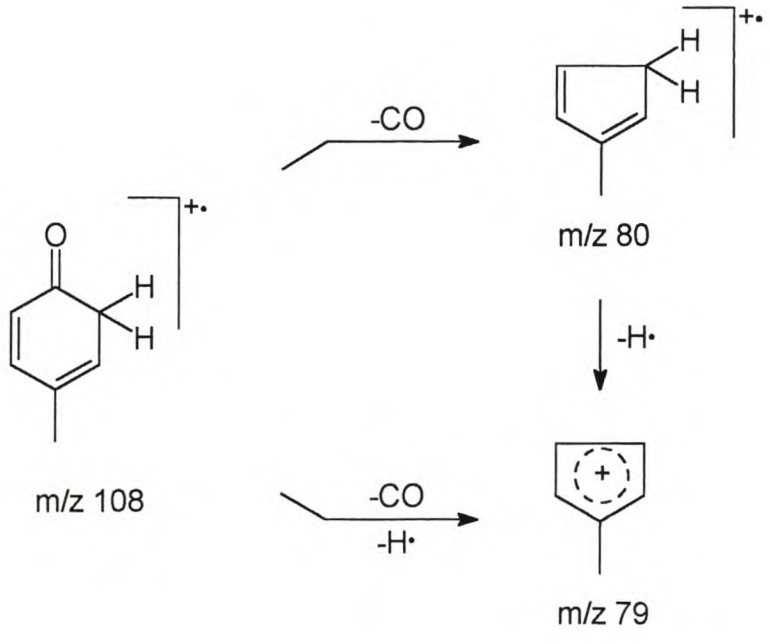
The EI mass spectra of components 2432 (Fig. 2.20) and 2440 (Fig. 2.21) both have prominent ions at m/z 77, 79, 107 and 108. The ion at m/z 107 is the base peak in the spectrum of component 2432 and m/z 108 is the molecular ion, and for component 2440 the ion at m/z 108 is both the base peak and the molecular ion. The

presence of these ions in the mass spectra under discussion and the general appearance of the spectra with the most abundant ions appearing in the higher mass range were construed as evidence that these components are cresols. Injection of synthetic *o*-, *m*-, and *p*-cresol individually, showed that *o*-cresol eluated first, followed by *p*- and *m*-cresol approximately 3 minutes later. Of the latter two, *p*-cresol eluated first, followed eight seconds later by *m*-cresol. This indicated that components 2432 and 2440 could be *p*- and *m*-cresol, respectively. Co-injection of the natural material with synthetic cresols confirmed this. The mass spectra of the cresols exhibit very strong $[M-1]^+$ peaks, presumably due to the formation of hydroxytropylium ions[15]:

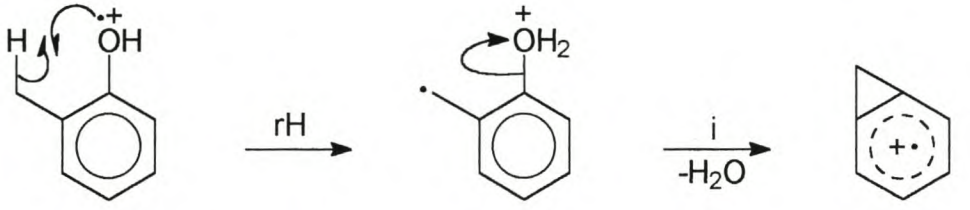


m/z 108 m/z 107

The apparent mechanism for the formation of the $[M-CO]^{+\cdot}$ ion at m/z 80, which is usually accompanied by the $[M-CHO]^+$ ion at m/z 79, involves elimination of the oxygen atom with its adjacent ring carbon. Deuterium-labelling studies indicate that CHO elimination is accompanied by substantial hydrogen scrambling[18]:



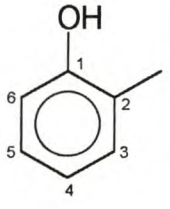
Ions corresponding to the elimination of water from the molecular ion become noticeable, and increase in abundance in the order *para* < *meta* < *ortho*[19]:

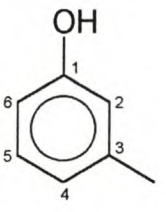


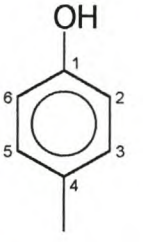
m/z 108 m/z 90

i = Inductive rearrangement

The EI mass spectra of components 2815 (Fig. 2.22) and 2825 (Fig. 2.23) are almost identical and exhibit many similarities to those of *p*- and *m*-cresol, components 2432 (Fig. 2.20) and 2440 (Fig. 2.21), respectively. The main difference between these two pairs of components is that components 2815 and 2825 both have a molecular ion at m/z 122, which is 14 atomic mass units higher than that of the cresols at m/z 108. If it is assumed that components 2815 and 2825 are both cresol-type compounds with an additional methyl group substituted either on the aromatic ring or on the methyl group already present, the following nine compounds are possible candidate structures:



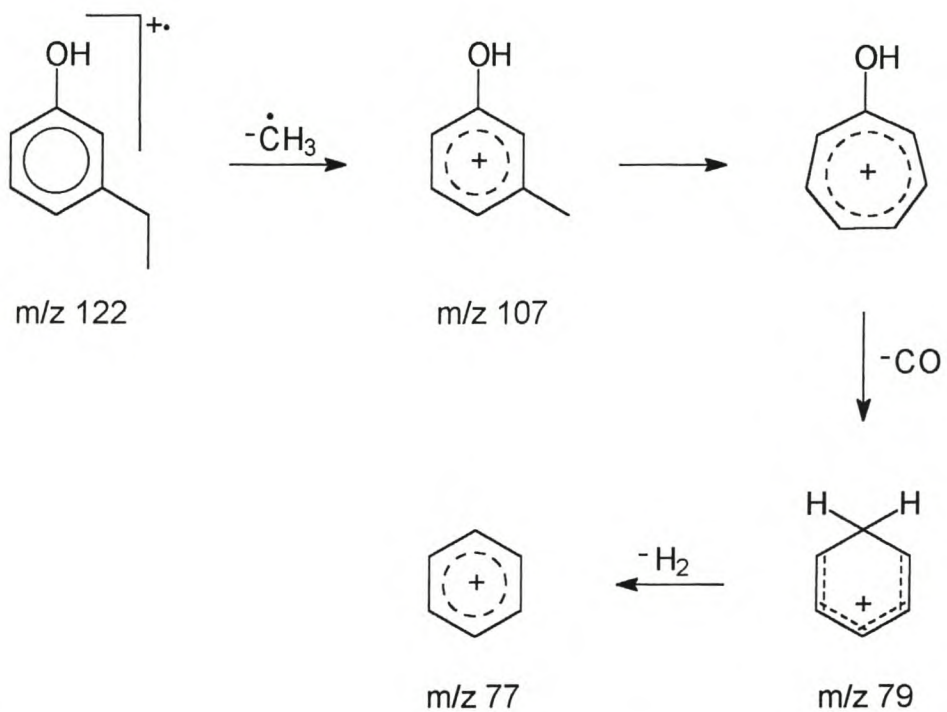




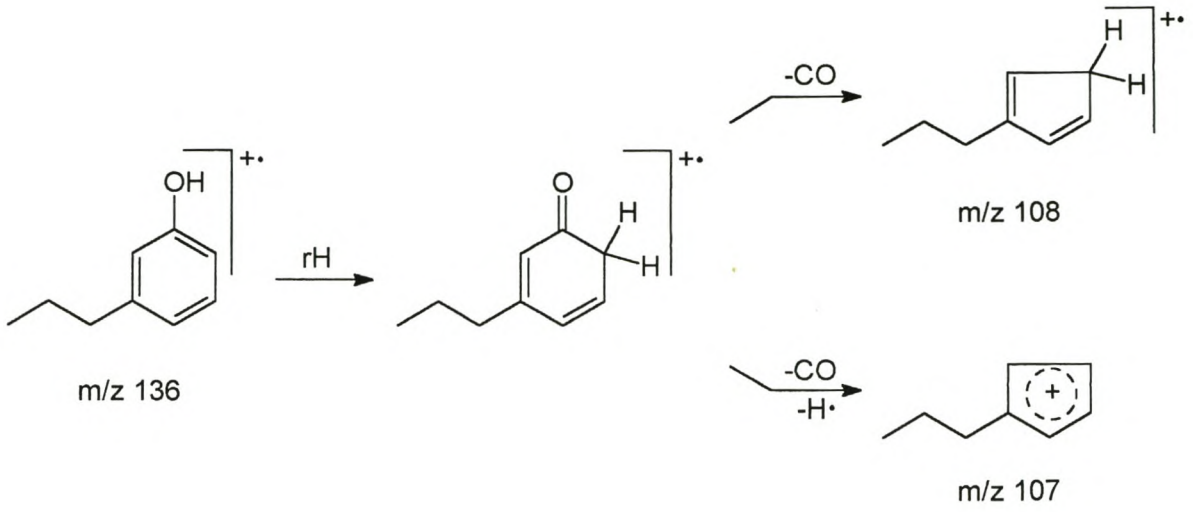
Derivatives of *o*-cresol	Derivatives of *m*-cresol	Derivatives of *p*-cresol
2,5-Dimethylphenol	2,3-Dimethylphenol	2,4-Dimethylphenol
2,6-Dimethylphenol	3,5-Dimethylphenol	3,4-Dimethylphenol
2-Ethylphenol	3-Ethylphenol	4-Ethylphenol

A study of the mass spectra of these nine compounds[13] showed that the m/z 122 molecular ion of the dimethylphenols has a relative abundance of 50% or more, while

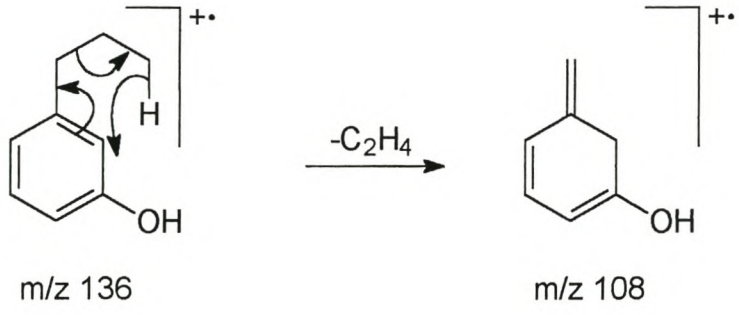
it is less than 50% for the ethylphenols. This prompted the retention-time testing of 2-, 3-, and 4-ethylphenol, since the m/z 122 ion in the mass spectra of components 2815 and 2825 have a relative abundance of 32% and 45%, respectively. Co-injection of these three commercially available compounds individually with the natural extract, showed that component 2815 is 4-ethylphenol and component 2825 is 3-ethylphenol. The most prominent ions in the mass spectra of these two components are explained using 3-ethylphenol as example:



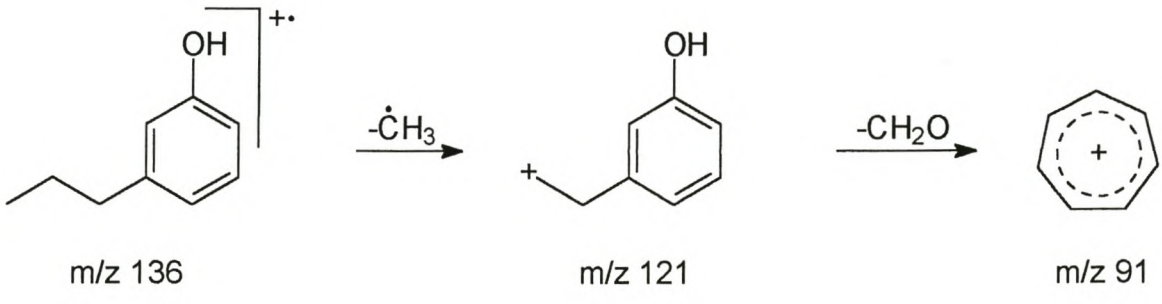
The EI mass spectrum of component 3181 (Fig. 2.24) displays the familiar aromatic compound ions at m/z 77, 79, 91, 107 (base peak), 108, 121 and 136 (molecular ion). A computer search gave 3-propylphenol with a probability of 94.8% and considering that phenol, *p*- and *m*-cresol, and 4- and 3-ethylphenol had already been found in the secretion, it was logical to assume that component 3181 could be a propylphenol. Co-injection experiments with the natural extract proved that this component is 3-propylphenol. The $[M\text{-}CO]^{+\cdot}$ and $[M\text{-}CHO]^{+}$ ions in the spectrum of this compound are distinctive for a phenol[20]:



The presence of the m/z 108 ion can also be explained in terms of a McLafferty rearrangement:

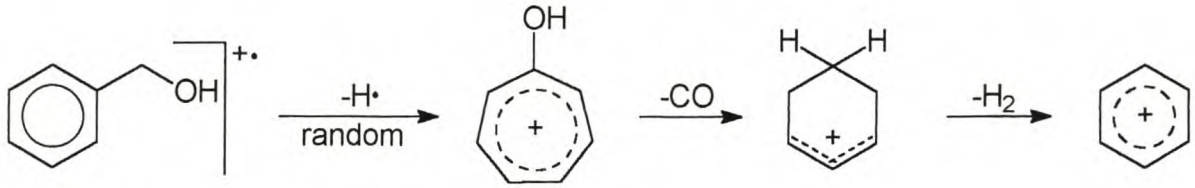


The ion at m/z 121 is formed through loss of a methyl group from the molecular ion, which leads to the formation of the tropyllium ion at m/z 91[21]:



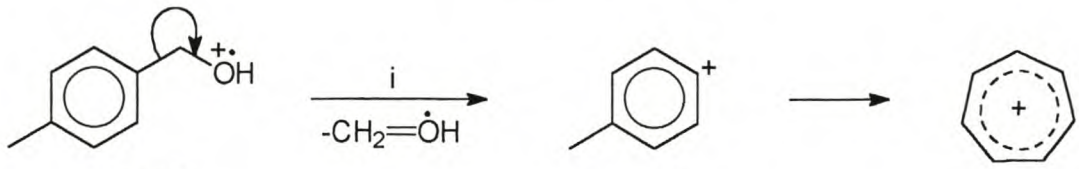
The EI mass spectrum of component 1992 (Fig. 2.25) exhibits two sets of very prominent ions at m/z 77 and m/z 79 (base peak), and at m/z 107 and m/z 108 (molecular ion), respectively. The obvious similarity between this mass spectrum and

the mass spectra of *p*- and *m*-cresol, components 2432 and 2480, respectively, led to the conclusion that component 1992 could be an aromatic isomer of the cresols. Component 1992, however, cannot be *o*-cresol, because the difference in retention time between *o*-cresol and *m*- and *p*-cresol is approximately 3 minutes, whereas there is a 7.5 minute difference in retention time between component 1992 and these cresols. It was therefore concluded that component 1992 could only be benzyl alcohol. Co-injection with commercially available benzyl alcohol confirmed this assumption. The prominent ions in the mass spectrum of benzyl alcohol, component 1992, can be explained as follows:



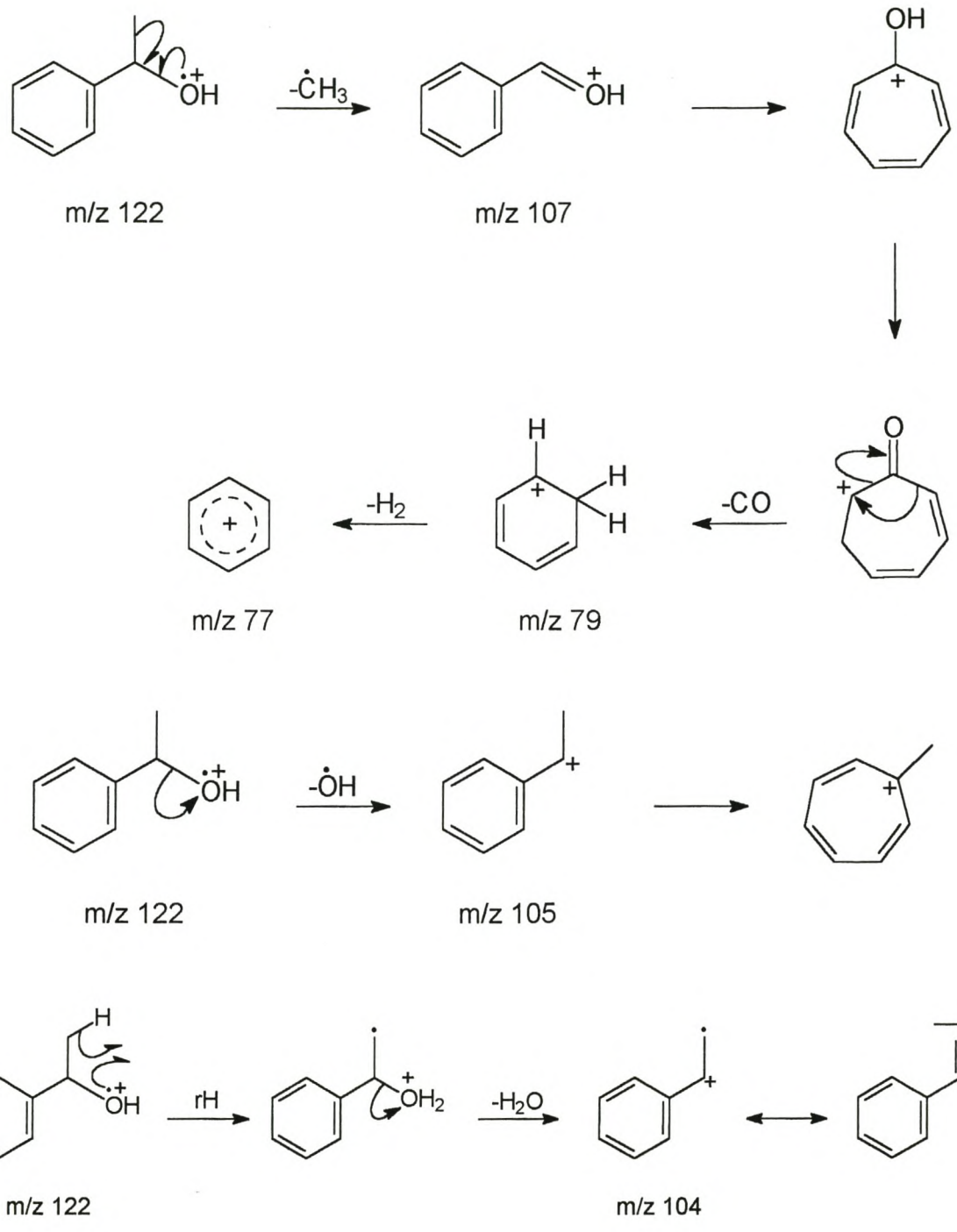
m/z 108 m/z 107 m/z 79 m/z 77

The EI mass spectrum of component 2028 (Fig. 2.26) has prominent ions at m/z 77, 79, 107 (base peak) and 122 (molecular ion). These ions are indicative of an aromatic system. The ion at m/z 107 can be ascribed to the elimination of a methyl group $[M\text{-}CH_3]^+$ and upon closer inspection $[M\text{-}OH]^+$ and $[M\text{-}H_2O]^{+\cdot}$ ions at m/z 105 and m/z 104, respectively, could be identified. If the mass spectrum of component 2028 is compared to that of benzyl alcohol (component 1992, Fig. 2.25), it can be seen that their molecular ions differ by 14 atomic mass units. It could then be hypothesized that component 2028 is a benzyl alcohol compound with one of the aromatic hydrogen atoms substituted by a methyl group, as, for example, in 2-methylbenzyl alcohol, or with one of the side-chain hydrogen atoms substituted by a methyl group, thus giving 1-phenylethanol. The absence of a tropyllium ion at m/z 91 in the mass spectrum of component 2028, however, excluded 2-, 3-, and 4-methylbenzyl alcohol as possible candidates:

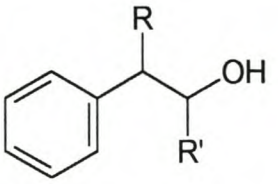


m/z 122 m/z 91

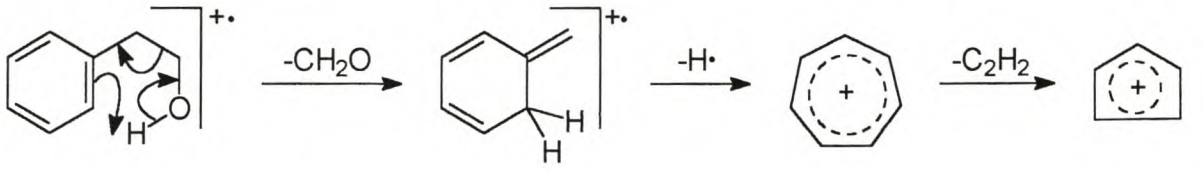
This left 1-phenylethanol as a possibility, which was confirmed by co-injection of the commercially available compound with the natural extract. The formation of the prominent ions in this mass spectrum can be explained as follows:



The EI mass spectrum of component 2299 (Fig. 2.27) has prominent ions at m/z 91 (base peak), 92 and 122. This is characteristic for 2-phenylethanol and some of its derivatives[22]:

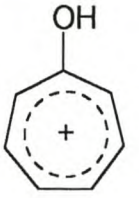


Since the molecular ion of component 2299 appeared to be at m/z 122, 2-phenylethanol (R = R' = H) was considered to be a likely structure. This assumption was confirmed by co-injection of synthetic 2-phenylethanol with the natural secretion. The ions at m/z 65, 91 and 92 are formed as follows:



m/z 122 m/z 92 m/z 91 m/z 65

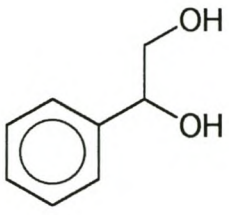
The EI mass spectrum of component 3485 (Fig. 2.28) displays similarities to those of benzyl alcohol (component 1992, Fig. 2.25) and 1-phenylethanol (component 2028, Fig. 2.26), discussed previously. It has the typical aromatic ions at m/z 77, 79, 91, 107 (base peak) and 138 (molecular ion). If the ion at m/z 107 is again taken to be the species $[C_7H_7O]^+$, shown below, and if the loss of 31 atomic mass units from m/z 138 to give m/z 107 is due to the loss of $[CH_2{=}OH]^{\cdot}$, component 3485 should have the molecular formula $C_8H_{10}O_2$, corresponding to a molecular mass of 138 Da.



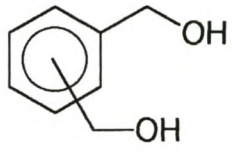
m/z 107

If component 3485 is then assumed to be either benzyl alcohol substituted with either a CH_2OH-group or with CH_3- and OH-groups, or 1-phenylethanol substituted with an OH-group, the following compounds are possible:

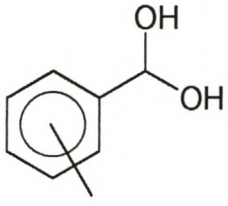
Group I



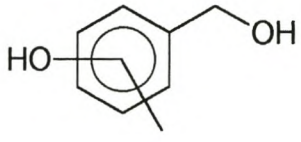
Group II



Group III

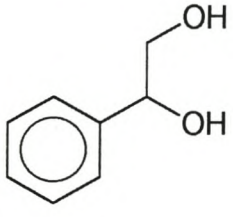


Group IV

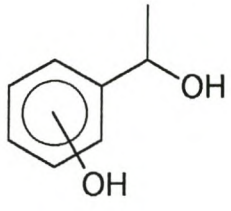


Derivatives of benzyl alcohol

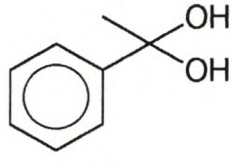
Group V



Group VI

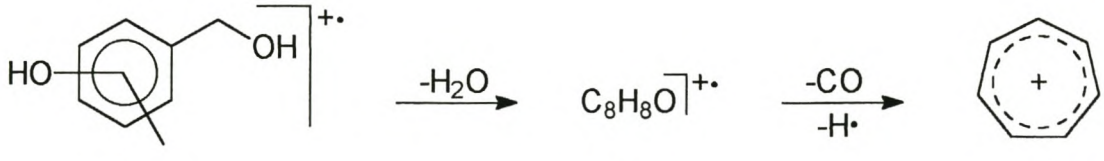


Group VII



Derivatives of 1-phenylethanol

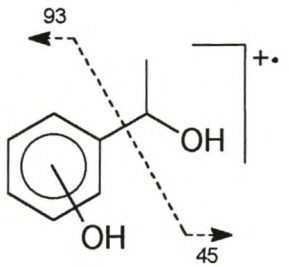
Compounds belonging to groups III and VII do not exist because they would eliminate water to give the corresponding carbonyl compounds. The mass spectra of the compounds of group IV all have m/z 91 and m/z 120 ions with relative abundance of more than 50%, whereas these ions in component 3485 have relative abundance of less than 30%[13]:



m/z 138 m/z 120 m/z 91

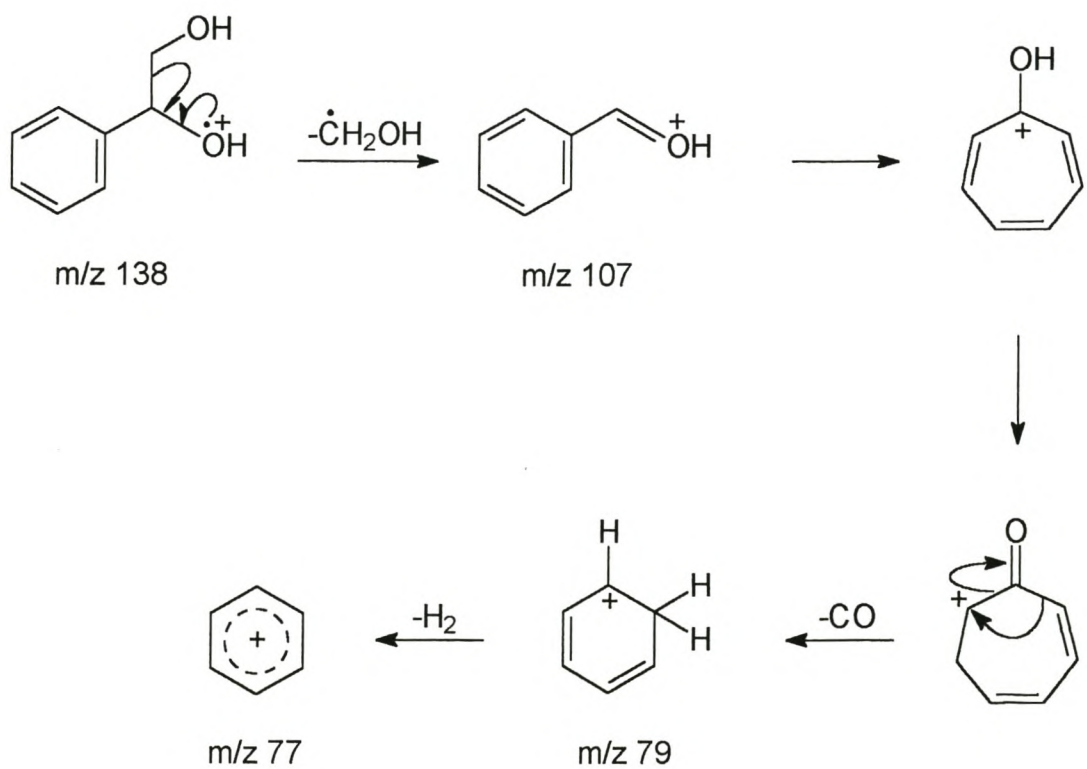
Group IV

The mass spectra of the compounds of group VI should all have abundant m/z 45 and m/z 93 ions[13]:



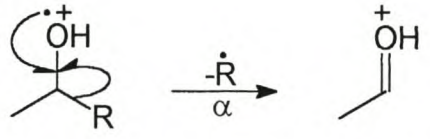
Group VI

This leaves groups I (=V) and II, and 1-phenyl-1,2-ethanediol (styrene glycol) [group I (=V)] was considered first. Component 3485 was identified as this diol by co-injection of the natural extract with synthetic 1-phenyl-1,2-ethanediol. The most prominent ions in the mass spectrum of 1-phenyl-1,2-ethanediol can be explained as follows:



2.2.8 Alcohols: Diols

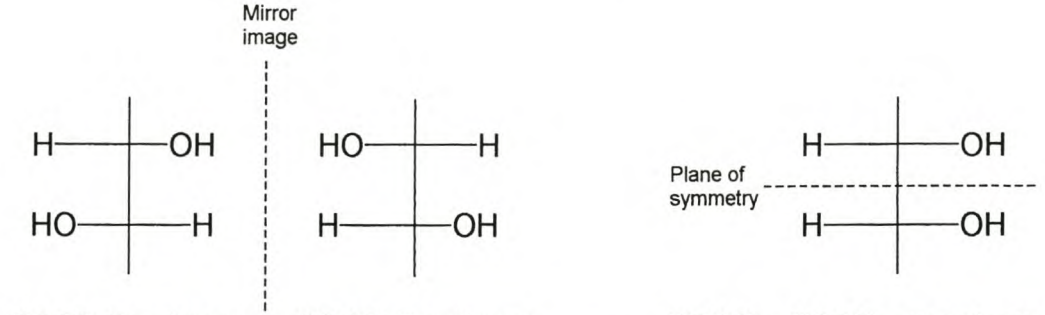
The EI mass spectra of components 949 (Fig. 2.29) and 988 (Fig. 2.30) are very similar and have base peaks at m/z 45. This could possibly be the result of an α-cleavage of a secondary methylcarbinol as follows:



m/z 90 m/z 45

(R > CH_3)

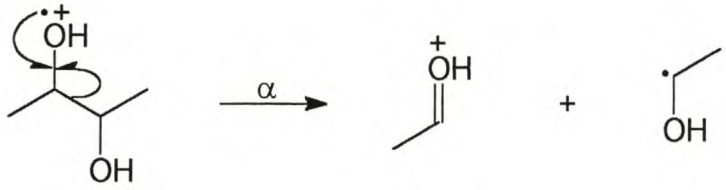
If the ion at m/z 90 in both spectra is accepted as the molecular ion, it is apparent that with 45 atomic mass units, R cannot be an alkyl group. It could, however, be C_2H_7N, COOH, CH_2CH_2OH or $CH(OH)CH_3$. The nitrogen containing fragment C_2H_7N can be discarded because the components have an even molecular mass at m/z 90, and therefore do not contain one nitrogen atom, leaving the compounds lactic acid, 1,3-butanediol and 2,3-butanediol as possibilities. Since components 949 and 988 have such similar mass spectra and retention times, 2,3-butanediol was considered first. The reason for this being that 2,3-butanediol has two chiral centres and a plane of symmetry, which results in three stereoisomers, namely a pair of optically active enantiomers and an optically inactive *meso*-isomer:



(2*S*,3*S*)-Enantiomer (2*R*,3*R*)-Enantiomer (2*R*,3*S*) = (2*S*,3*R*) - *meso*-isomer

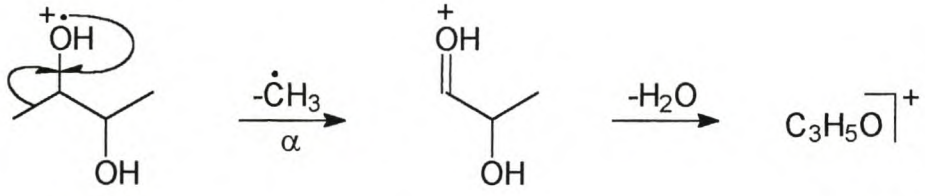
The *meso*-isomer is diastereomerical with the pair of enantiomers and therefore they have similar but not identical chemical properties and different physical properties. This results in a retention time for the pair of enantiomers that differ from that of the

meso-isomer. Co-injection of this diol and the secretion showed that 2,3-butanediol co-eluated with components 949 and 988 and since the *meso*-isomer has a slightly higher boiling point than the pair of enantiomers, it was assumed that component 949 represents one or both of the enantiomers that cannot be separated on an achiral column, and that component 988 is the *meso*-isomer. The prominent ions in the mass spectra of these compounds can be explained as follows:



m/z 90 m/z 45 (100%)

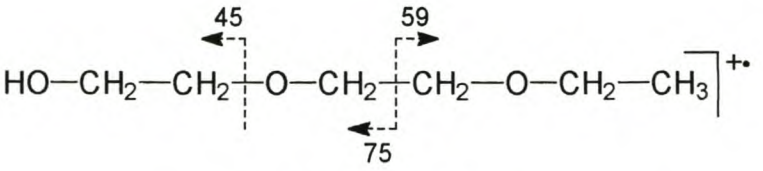
Loss of the larger group is favoured, explaining why m/z 45 (base peak) has a higher relative abundance than m/z 75 (7.4%):



m/z 90 m/z 75 (7.4%) m/z 57

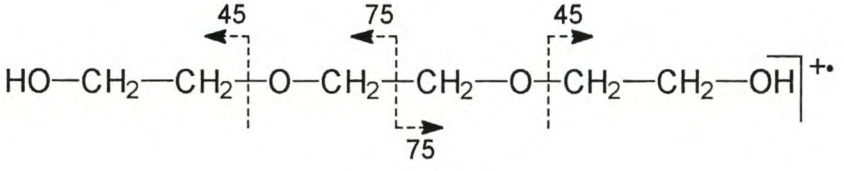
2.2.9 Alcohols: Oligoethylene glycols

Although component 1664 has two prominent ions at m/z 45 (base peak) and m/z 59 (34%) in its EI mass spectrum (Fig. 2.31), the absence of a molecular ion and the presence of a possible rearrangement ion at m/z 72 (28%), of which the mechanism could not be explained, made any reasonable deductions almost impossible. Using these three ions and their relative abundance in a computer search[13], yielded di(ethylene glycol)monoethyl ether as a possibility and co-injection of the commercially available compound with the natural extract resulted in co-elution of di(ethylene glycol)monoethyl ether with component 1664. The ions at m/z 45, 59 and 75 can be explained as follows:

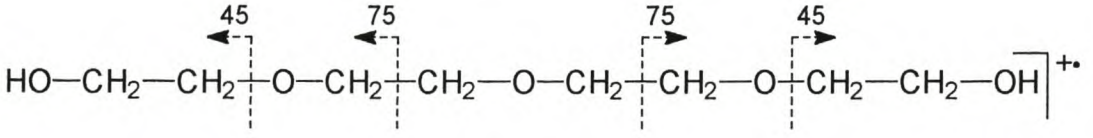


$C_6H_{14}O_3$, 134 Da

The EI mass spectra of components 3081 (Fig. 2.32) and 4180 (Fig. 2.33) are similar in that they both have a very prominent ion at m/z 45 (base peak). This is indicative of 2-alkanols, but a computer search[13] gave tri(ethylene glycol) and tetra(ethylene glycol) as possibilities for components 3081 and 4180, respectively. Co-injection of a mixture of these two synthetic glycols with the secretion showed that tri(ethylene glycol) co-eluated with component 3081 and tetra(ethylene glycol) co-eluated with component 4180. The ions at m/z 45 and m/z 75 in the mass spectra of these compounds can be explained, respectively, as follows:



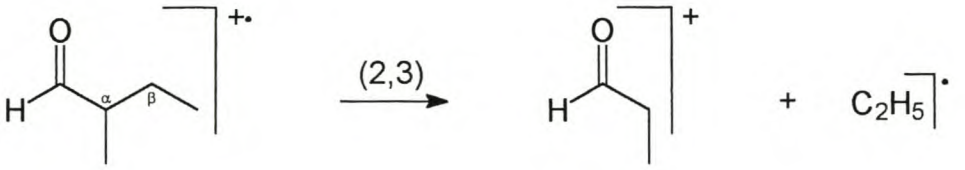
$C_6H_{14}O_4$, 150 Da



$C_8H_{18}O_5$, 174 Da

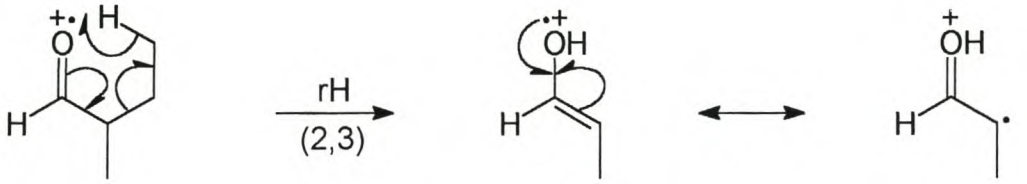
2.2.10 Aldehydes: Aliphatic (Saturated)

The EI mass spectrum of component 298 (Fig. 2.34) has a base peak at m/z 44, and if the ion at m/z 86 is assumed to be the molecular ion and the ions at m/z 68 and m/z 58 therefore $[M\text{-}H_2O]^{+\cdot}$ and $[M\text{-}C_2H_4]^{+\cdot}$, respectively, component 298 could be an aliphatic aldehyde. If this component were 2-methylbutanal, the base peak would have been at m/z 57, which is due to a β-cleavage[13]:



m/z 86 m/z 57

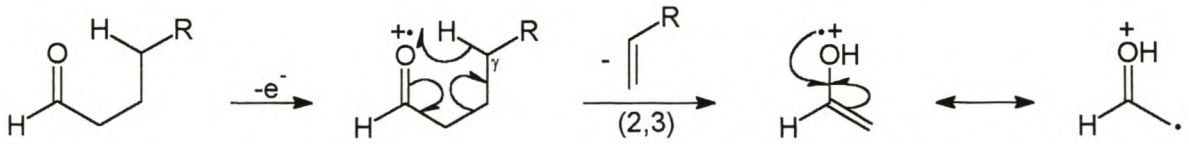
This is surprising, because a McLafferty rearrangement, giving an m/z 58 ion as base peak, would have been anticipated:



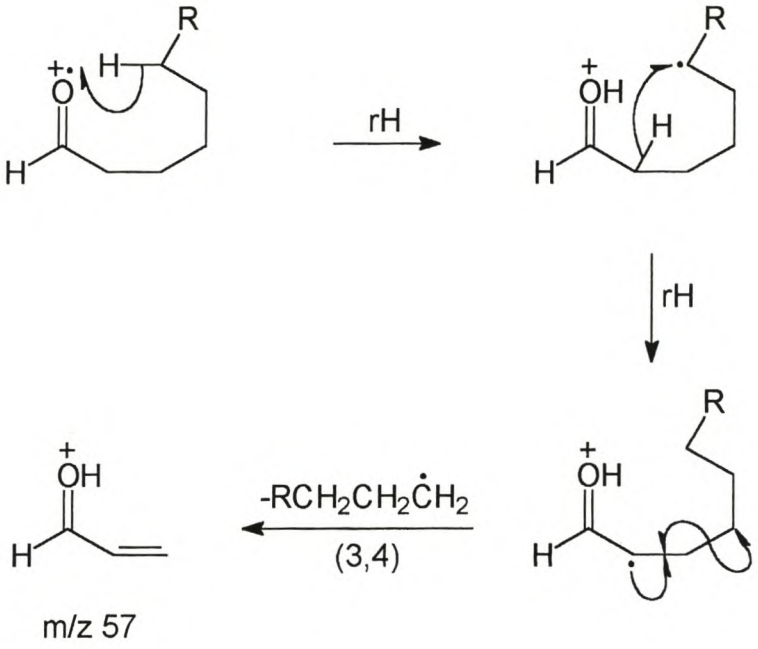
m/z 86 m/z 58

2,2-Dimethylpropanal does not give an m/z 44 McLafferty rearrangement ion due to the absence of a γ-hydrogen atom. This leaves two possibilities, namely pentanal (valeraldehyde) and 3-methylbutanal (isovaleraldehyde). Retention-time testing of these two synthetic compounds showed that the retention time of pentanal was too long while co-injection of 3-methylbutanal with the natural extract confirmed that component 298 is 3-methylbutanal.

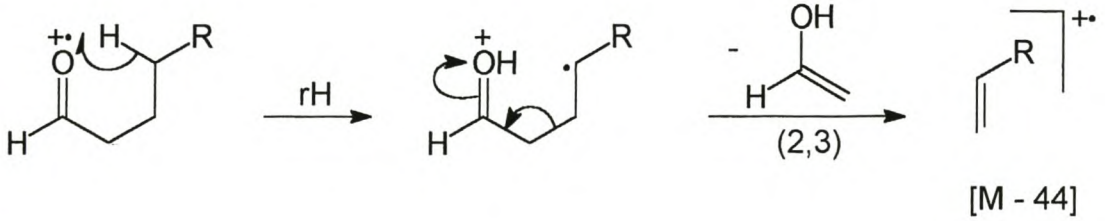
The EI mass spectra of components 1909 (Fig. 2.35), 3640 (Fig. 2.36) and 4021 (Fig. 2.37) all have prominent ions at m/z 44 and m/z 57, and they exhibit clusters of ions, 14 atomic mass units apart, at m/z 29, 43, 57, 85, *etc.*, which can be ascribed to the formation of $[C_nH_{2n+1}CO]^+$ and $[C_nH_{2n+1}]^+$ ions. This is characteristic of unbranched aliphatic aldehydes[23]. Since unbranched aldehydes, containing four to seven carbon atoms, have base peaks at m/z 44[24], it was assumed that these components were aldehydes with more than eight carbon atoms. The ion at m/z 44 must be a rearrangement peak as it occurs at an even mass, and can be attributed to the characteristic McLafferty rearrangement, resulting in the elimination of an olefin:



The accompanying [McLafferty + 13] ion at m/z 57 is formed as follows:

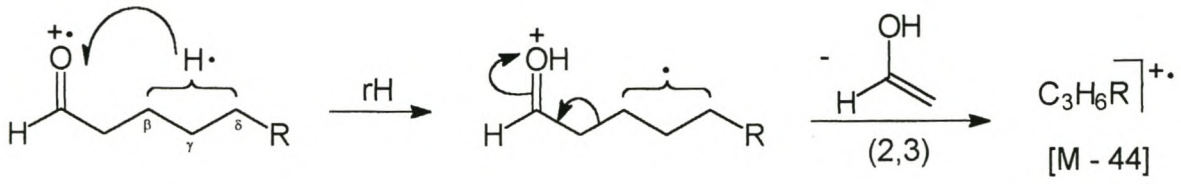


Another characteristic feature of aldehydes without α-branching, is the presence of an ion at $[M-44]^{+\cdot}$. Labelling experiments with ^{18}O show that fragments resulting from this loss are ions with the general formula $[C_nH_{2n}]^{+\cdot}$. This implies β-cleavage with hydrogen atom transfer as in a McLafferty rearrangement, but with charge-retention on the alkene fragment[25]:

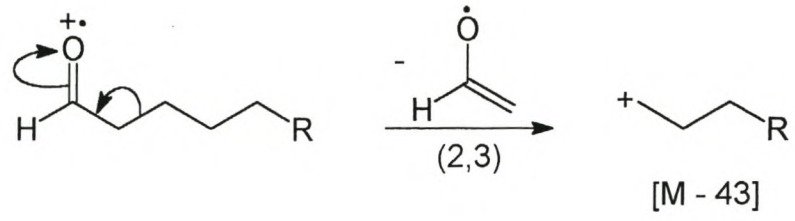


Data obtained with deuterated hexanals, however, demonstrate that this process is in fact not of the site-specific McLafferty rearrangement type[26]. Of the total transfer of

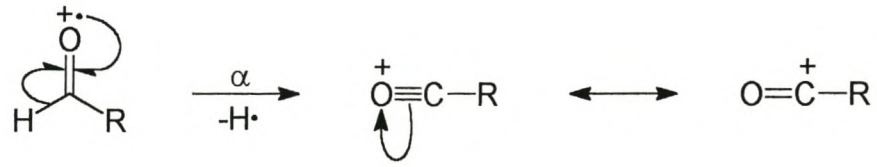
hydrogen atoms, 81% originate from the β-, γ- or δ-positions, and therefore the following formulation is more realistic:

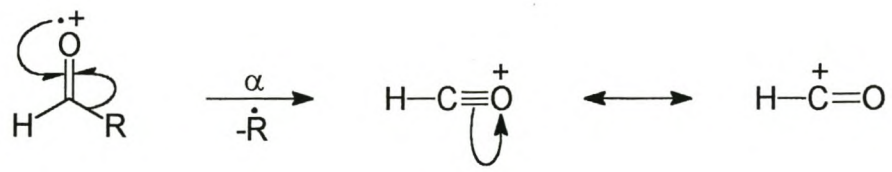


Another type of β-cleavage also occurs in which no hydrogen atom transfer takes place and the positive charge is retained on an alkyl fragment[27] with the general formula $[C_nH_{2n+1}]^+$:

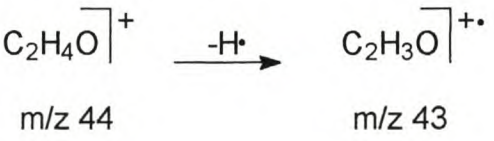


Both possible α-cleavage reactions occur, namely elimination of either an alkyl radical or a hydrogen atom, with the charge remaining on the oxygen-containing fragment. The loss of the larger alkyl radical is favoured. The formation of these ions can be rationalized as follows:

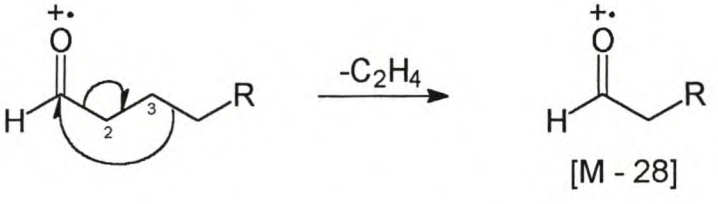




Another fairly prominent ion in the mass spectra of unbranched aldehydes appears at m/z 43. This ion has been shown to be an oxygen-containing fragment with elemental composition $[C_2H_3O]^+$, and evidence exists that it is formed as a result of the loss of a hydrogen atom from the m/z 44 ion[28]:



On the basis of the above, a series of synthetic unbranched aldehydes (octanal through tetradecanal, hexadecanal and octadecanal) were co-injected with the natural extract and components 1909, 3640 and 4021 were found to be nonanal, tridecanal and tetradecanal, respectively. The molecular ions of the larger aliphatic aldehydes often have a low abundance, but the presence of characteristic $[M-H_2O]^{+\cdot}$ and $[M-C_2H_4]^{+\cdot}$ fragments can be used for identification[29]. Deuterium-labelling experiments have shown that the major site of transfer for the hydrogen atoms in the elimination of water, is the C3 carbon atom. Data from deuterated hexanals indicate that the C2 and C3 carbon atoms are eliminated as a unit in the expulsion of ethylene[30]:

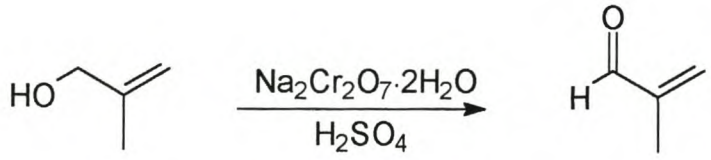


The $[M-H_2O]^{+\cdot}$ and $[M-C_2H_4]^{+\cdot}$ ions can be seen at m/z 124 and m/z 114, respectively, in the mass spectrum of nonanal (component 1909, Fig. 2.35).

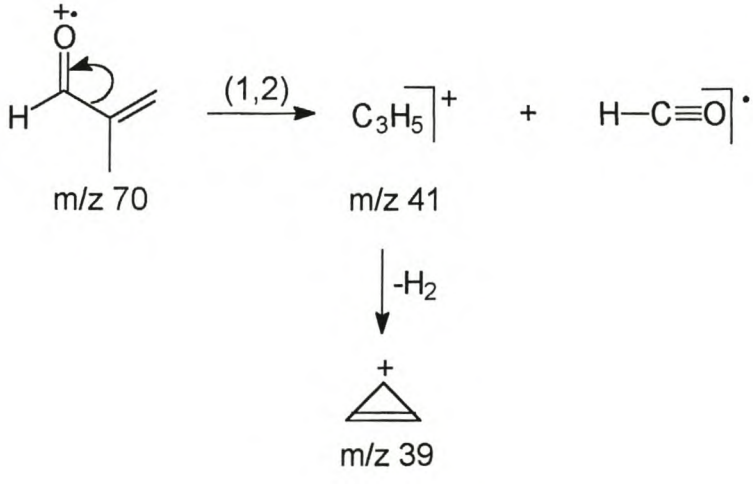
2.2.11 Aldehydes: Aliphatic (Unsaturated)

The EI mass spectrum of component 204 (Fig. 2.38) in the total ion chromatogram of the interdigital secretion of the black wildebeest (Fig. 2.1) has a prominent ion at m/z 70, which was assumed to be the molecular ion. The presence of both an $[M-1]^+$ ion at m/z 69 and an m/z 29 ion, which could indicate an $[HCO]^+$

ion, led to the assumption that this component was an unsaturated aliphatic aldehyde, with molecular formula C_4H_6O. The possibilities were therefore *cis*- and *trans*-2-butenal (crotonaldehyde), 3-butenal and 2-methyl-2-propenal (methacrolein). Injection of commercially available 2-butenal showed that the retention time was too long and it was assumed that this would also be the case for 3-butenal, which was not commercially available. 2-Methyl-2-propenal was synthesized according to the scheme shown below (see § 3.4.5 and mass spectrum Fig. 3.5) and by co-injection of the synthetic product with the natural interdigital extract, component 204 was positively identified as 2-methyl-2-propenal.



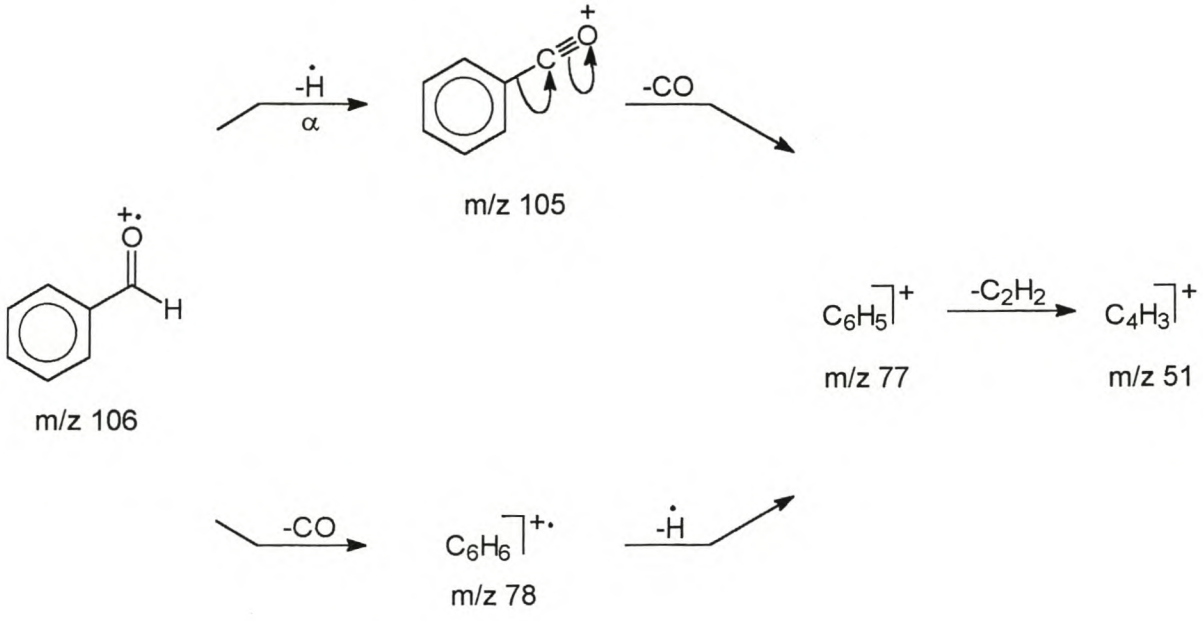
The ions at m/z 39 and m/z 41 can be explained as follows:



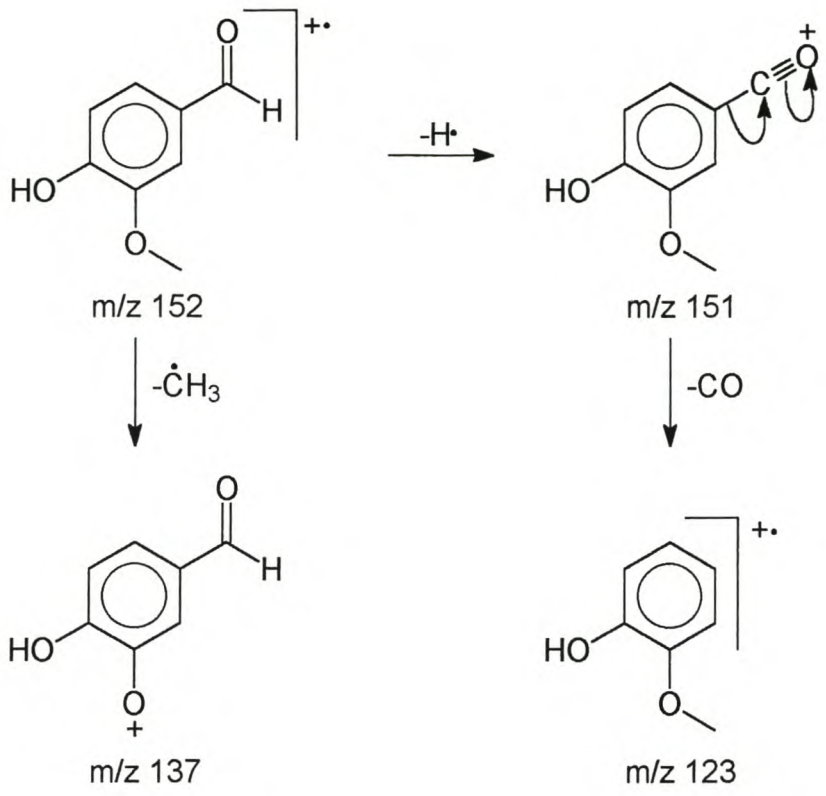
2.2.12 Aldehydes: Aromatic

The EI mass spectrum of component 1413 (Fig. 2.39) has prominent ions at m/z 51, 77 (base peak), 78, 105 and 106 (molecular ion). The two ions at m/z 105 and m/z 106 are of almost identical relative abundance, indicating that component 1413 could be benzaldehyde[31]. Co-injection of synthetic benzaldehyde with the

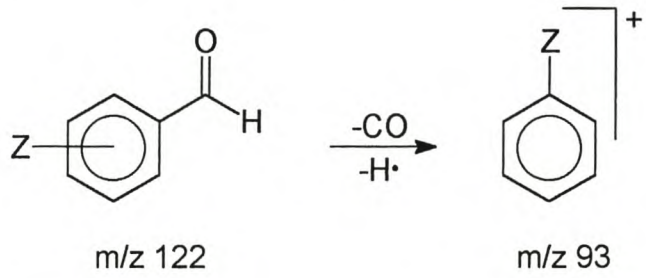
natural extract confirmed that component 1413 is in fact benzaldehyde. The prominent ions in its mass spectrum can be explained as follows:



The EI mass spectrum of component 3769 (Fig. 2.40) shows a very prominent pair of ions at m/z 151 (base peak) and m/z 152 (molecular ion, 94.6%), the relative abundance of which corresponds to that of the pair of ions at m/z 105 and m/z 106 in the mass spectrum of benzaldehyde (component 1413, Fig. 2.39). It was therefore assumed that component 3769 is a substituted benzaldehyde. A computer search using the Wiley library[13] gave 4-hydroxy-3-methoxybenzaldehyde (vanillin) and 3-hydroxy-4-methoxybenzaldehyde (isovanillin) as possibilities. Co-injection of commercially available 4-hydroxy-3-methoxybenzaldehyde with the secretion proved that component 3769 is in fact vanillin. The prominent ions in the spectrum of this compound at m/z 152, 151, 137 and 123 can be explained as follows:

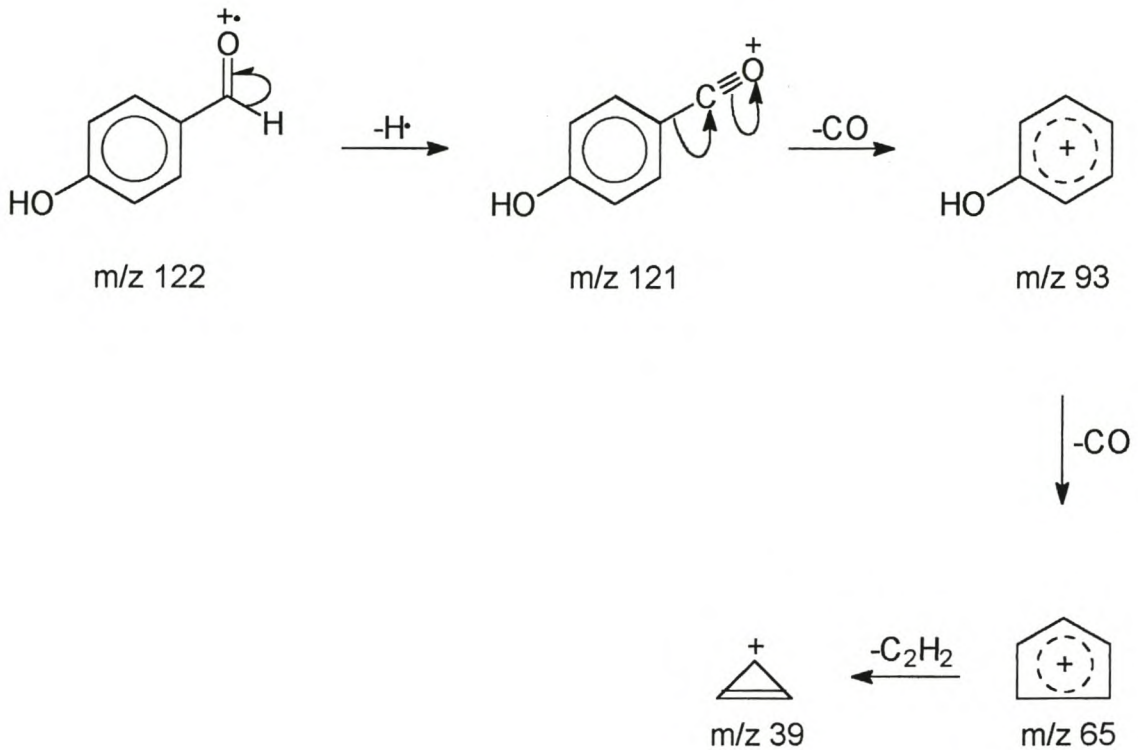


As was seen in the previous two cases, the EI mass spectrum of component 4220 (Fig. 2.41) also displays two very prominent ions, with comparable relative abundance, this time at m/z 121 (base peak) and m/z 122 (molecular ion, 90.3%). If this is again taken as an indication that component 4220 is a substituted benzaldehyde, the formation of the $[M-29]^+$ ion can be explained as follows:

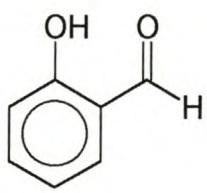


The molecular structure of the ion at m/z 93 is C_6H_4Z; Z must therefore be a hydroxyl group and component 4220 must be 2-, 3- or 4-hydroxybenzaldehyde. If the published mass spectra of these compounds are studied[13], it is seen that only 4-hydroxybenzaldehyde has a base peak at m/z 121; 2- and 3-hydroxybenzaldehyde have base peaks at m/z 122, and therefore the 4-isomer was considered as a likely candidate. Co-injection of commercially available 3- and 4-hydroxybenzaldehyde with

the natural extract resulted in co-eluation of the latter compound with component 4220. The prominent peaks at m/z 122, 121, 93, 65 and 39 in the mass spectrum of this compound can be explained as follows:

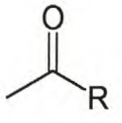


The EI mass spectrum of component 1789 (Fig. 2.42), appearing as a very small peak in the total ion chromatogram (TIC) was later found to have the same characteristic ions as component 4220; the only difference being that the ion at m/z 122 is the base peak in the case of component 1789. Using the previous discussion as a basis, this was taken as an indication that this component could be either 2- or 3-hydroxybenzaldehyde. Co-injection of synthetic samples of these two compounds with the natural interdigital extract proved that component 1789 is in fact 2-hydroxybenzaldehyde (salicylaldehyde):



2.2.13 Ketones: Aliphatic (Saturated)

The EI mass spectrum of component 314 (Fig. 2.43) has prominent ions at m/z 43 (base peak) and m/z 86 (molecular ion). It has been shown that m/z 43 $[CH_3CO]^+$ is the base peak in the mass spectra of a series of branched and unbranched methyl ketones[32] and since the molecular ion of ketones is usually observable[29], it was assumed that component 238 was a methyl ketone having the molecular formula $C_5H_{10}O$:



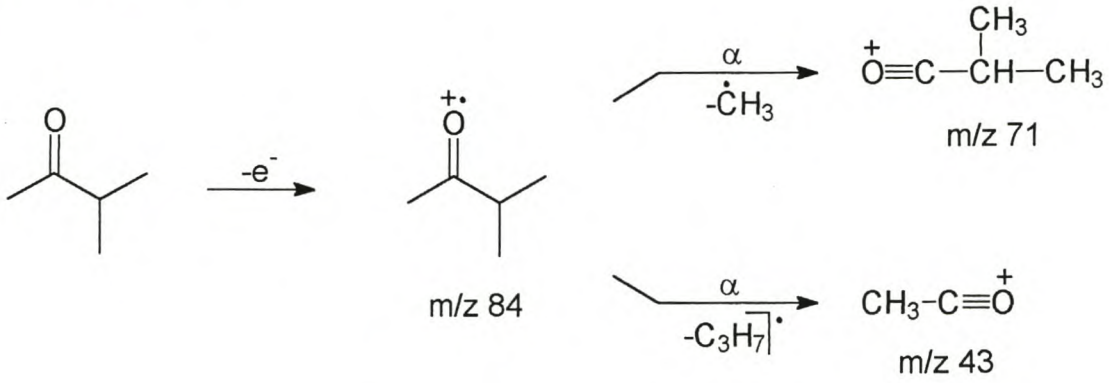
(R = C_3H_7)

When a chain of three or more carbon atoms is attached to the carbonyl group, McLafferty rearrangement (β-fission with transfer of a γ-hydrogen atom) becomes important[33]. However, the situation with ketones is more complex than with aldehydes for two reasons:

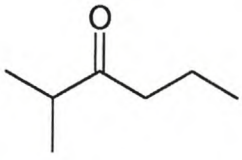
1. Even with unbranched ketones, the mass of the rearrangement ion will vary with the size of the alkyl group which is not involved in the rearrangement. Unbranched methyl ketones or those branched beyond the third carbon atom give the rearrangement ion at m/z 58. Ethyl and propyl or isopropyl ketones, with the same reservations as to the branching, give the ion at m/z 72 and m/z 86, respectively.
2. When there is a chain of three or more carbon atoms in each alkyl group, a second McLafferty rearrangement becomes possible, since the enolic product of primary rearrangement can again fragment through a six-membered state to give an m/z 58 ion.

When this is taken into consideration, it is clear that component 314 must be a branched ketone, because of the absence of a rearrangement ion at m/z 58. The only possibility that meets all of the criteria is 3-methyl-2-butanone and this was confirmed by co-injection of the commercially available compound with the natural

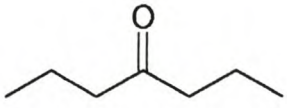
extract. The ions at m/z 43, 71 and 86 in the spectrum of this ketone can be explained as follows:



The EI mass spectrum of component 889 (Fig. 2.44) exhibits prominent ions at m/z 43 and m/z 71 and ions of lower abundance at m/z 114 (assumed to be the molecular ion), 58, and 86. The base peak at m/z 43 was taken as an indication that component 889 could probably be an alkanone, the m/z 86 ion as evidence in favour of the component being a propyl or isopropyl ketone and the m/z 58 ion as evidence of the presence of three or more carbon atoms in each alkyl group. The possibilities were therefore:

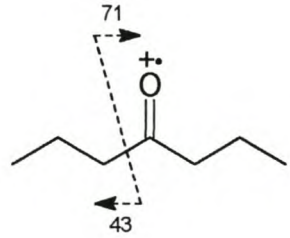


2-Methyl-3-hexanone



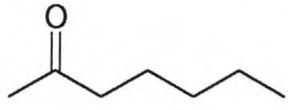
4-Heptanone

2-Methyl-3-hexanone, however, cannot produce an m/z 58 ion, because the isopropyl group does not have a γ-hydrogen atom to give a second McLafferty rearrangement. Co-injection of commercially available 4-heptanone with the natural extract proved that component 889 is 4-heptanone. The m/z 43 and m/z 71 ions are formed by α-cleavage as follows:

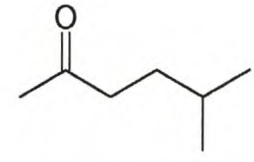


The EI mass spectra of components 983 (Fig. 2.45), 1269 (Fig. 2.46) and 1436 (Fig. 2.47) exhibit base peaks at m/z 43 and using the same arguments as before, these components were considered to be methyl ketones. The presence of the prominent m/z 58 ion indicates that these components have side-chains of three or more carbon atoms and that branching can only be at or beyond C4. Components 983 and 1436 have molecular ions at m/z 114 (a 2-heptanone) and m/z 128 (a 2-octanone), respectively, which means that the following candidate structures for components 983 and 1436 can be formulated:

Component 983 ($C_7H_{14}O$):

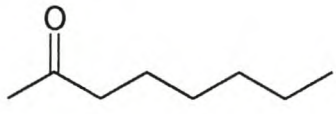


2-Heptanone

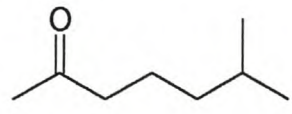


5-Methyl-2-hexanone

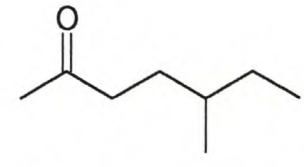
Component 1436 ($C_8H_{16}O$):



2-Octanone



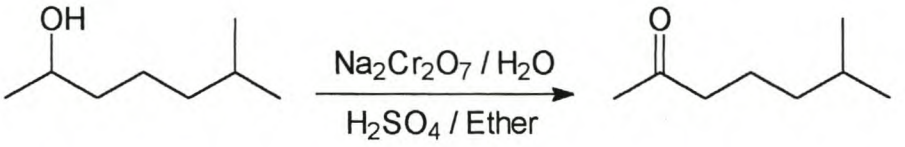
6-Methyl-2-heptanone



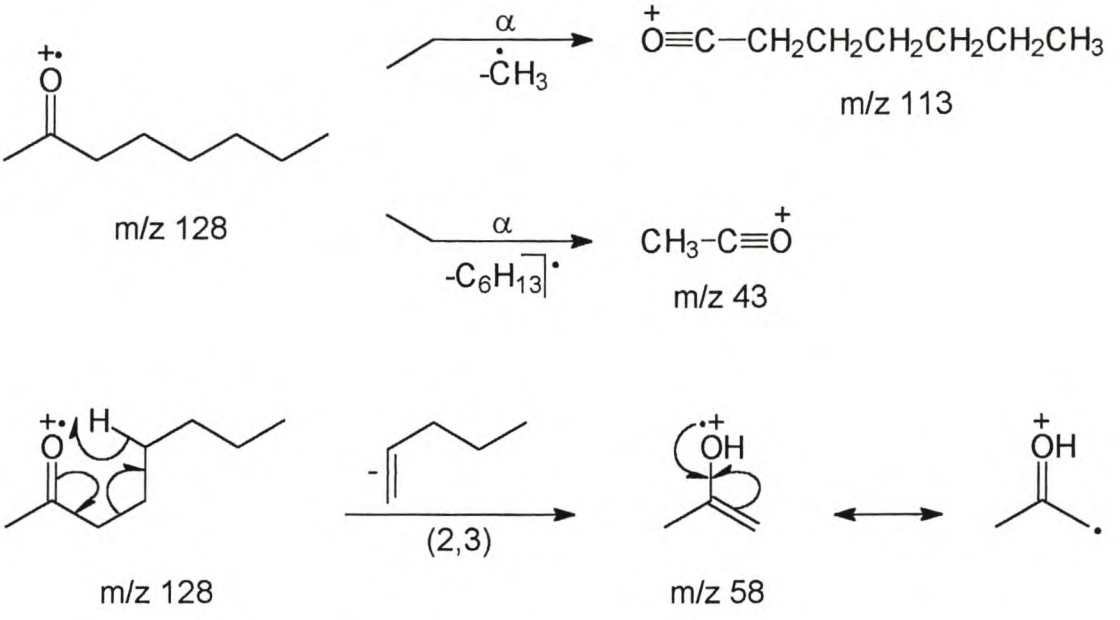
5-Methyl-2-heptanone

The unbranched ketones were considered first and co-injection of commercially available 2-heptanone and 2-octanone with the natural secretion confirmed that components 983 and 1436 are in fact 2-heptanone and 2-octanone, respectively. Component 1269 did not exhibit a molecular ion, but its retention time was between that of component 983 and 1436, so that is was thought to be a branched

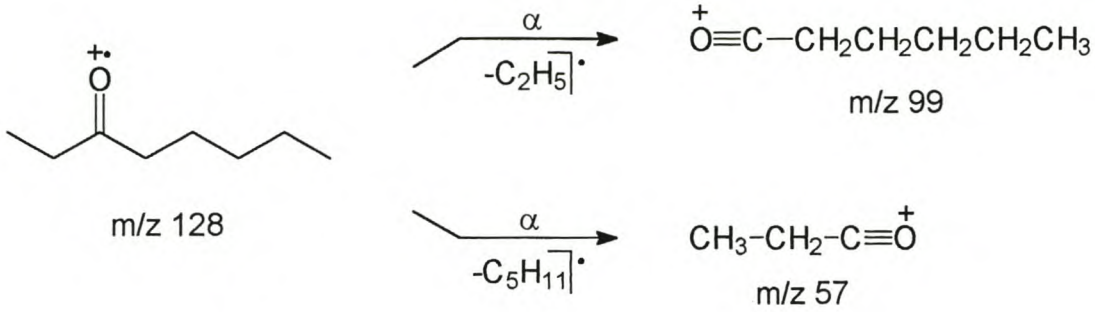
2-octanone. 6-Methyl-2-heptanone was synthesized first according to the scheme below (see § 3.4.6 and mass spectrum Fig. 3.6) and co-injection with the natural extract proved that component 1269 is this branched methyl ketone.

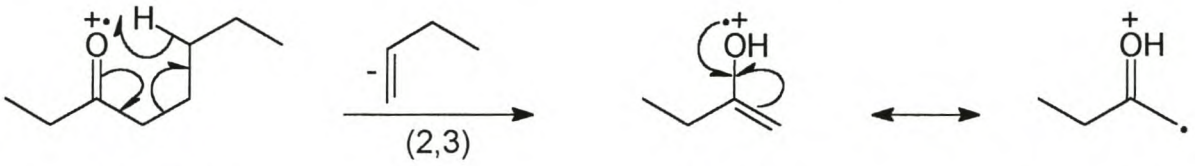


The prominent ions in the mass spectrum of 2-octanone can be explained as follows:



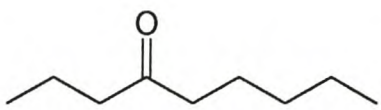
The EI mass spectrum of component 1169 (Fig. 2.48) has prominent ions at m/z 43, 57 (base peak), 71, 72, 99 and 128 (molecular ion), and an ion of low relative abundance at m/z 58. Component 1169 could therefore be a saturated octanone, but the low abundance (10.0%) of the m/z 58 ion excluded it from being a 2-octanone. Component 1169 could therefore be 3- or 4-octanone. The rearrangement ion at m/z 72 as well as the absence of an ion at m/z 85 that could be formed by α-cleavage, favours an ethyl ketone such as 3-octanone as possible structure. The identification of component 1169 as 3-octanone was confirmed by co-injection of the synthetic ketone with the natural extract. The prominent ions in the mass spectrum of 3-octanone, component 1169, can be explained as follows:



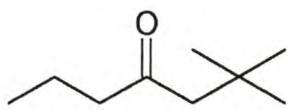


m/z 128 m/z 72

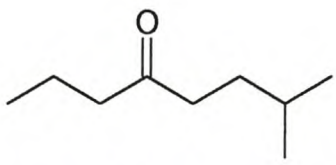
The EI mass spectrum of component 1766 (Fig. 2.49) exhibits ions at m/z 43 (base peak), 58, 71, 86, 99 and 142 (molecular ion). The presence of the ion at m/z 43, together with the molecular mass of the compound could indicate a methyl, propyl or isopropyl ketone, but the rearrangement ion at m/z 58 is only compatible with a methyl ketone (single rearrangement) or a propyl ketone (double rearrangement). The rearrangement ion at m/z 86, however, indicates that one of the alkyl groups must be a propyl group. The presence of an m/z 58 peak excludes the possibility of α-substitution, so that component 1766 must be one of the following[34]:



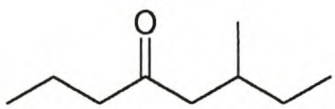
4-Nonanone



2,2-Dimethyl-4-heptanone



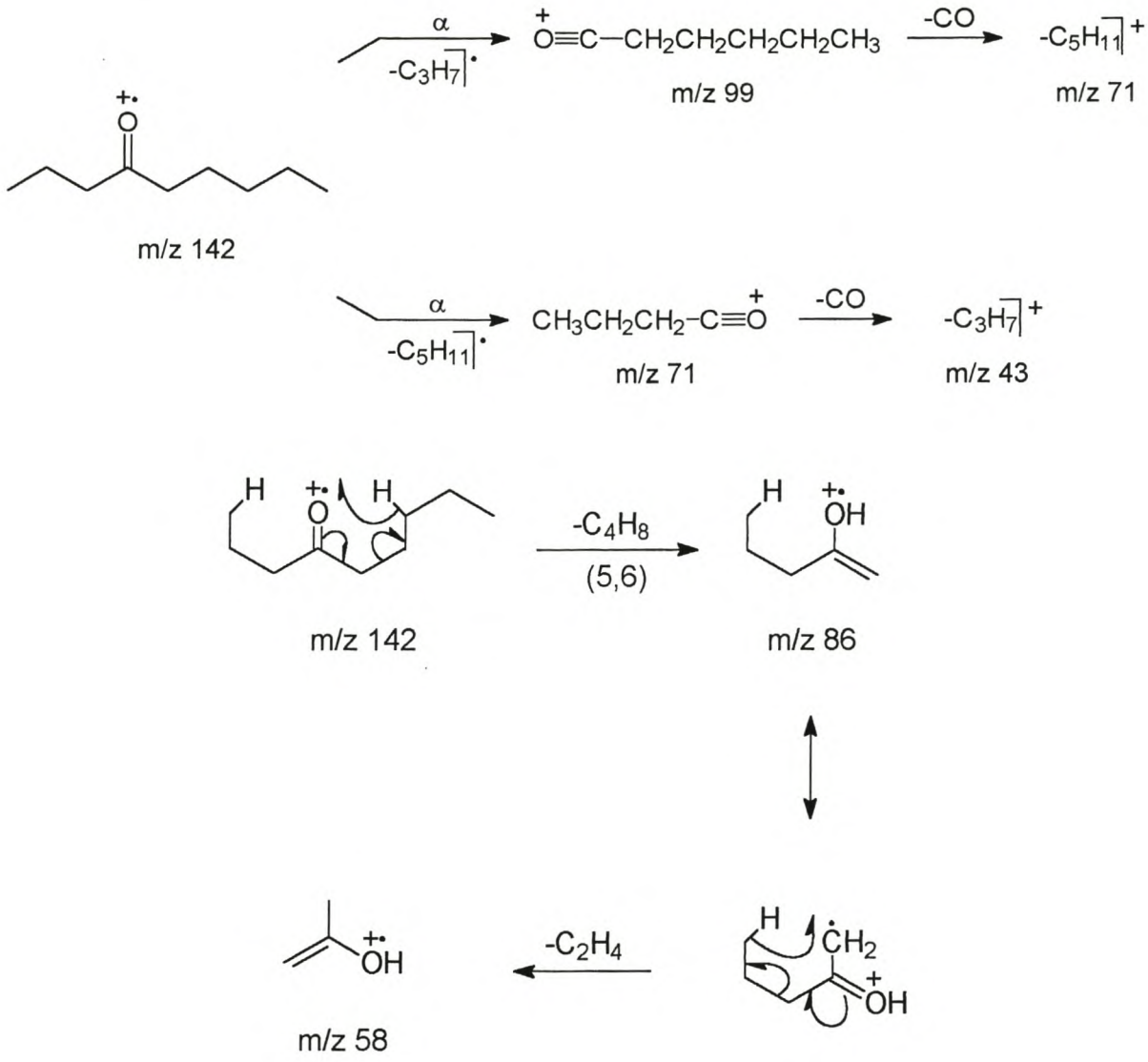
7-Methyl-4-octanone



6-Methyl-4-octanone

Since 4-nonanone had been found in the interdigital secretion of the bontebok, *Damaliscus dorcas dorcas*[35], and the blesbok, *Damaliscus dorcas phillipsi*[35], it was

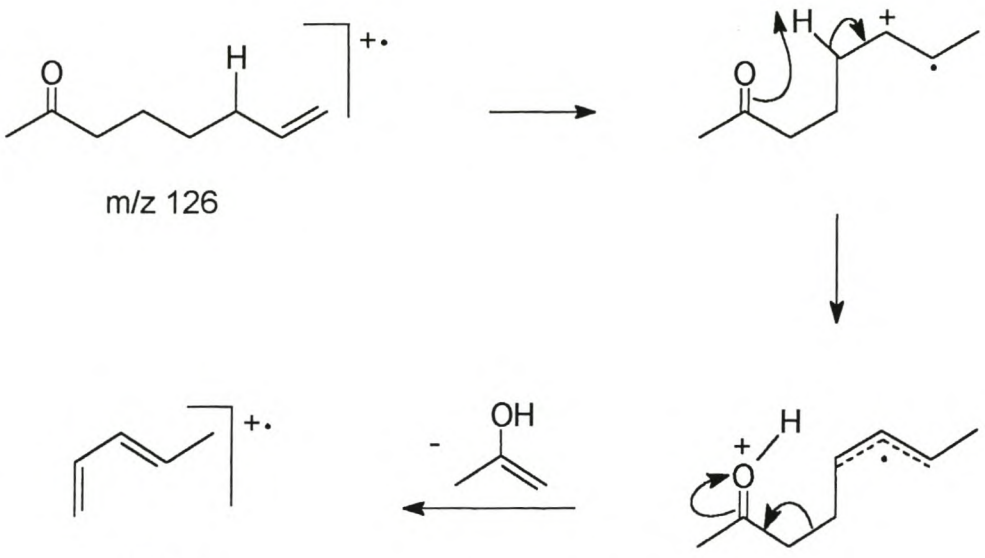
considered as the most likely structure. Co-injection of synthetic 4-nonanone with the natural secretion proved that component 1766 is 4-nonanone. The prominent ions in the mass spectrum of 4-nonanone can be explained as follows:



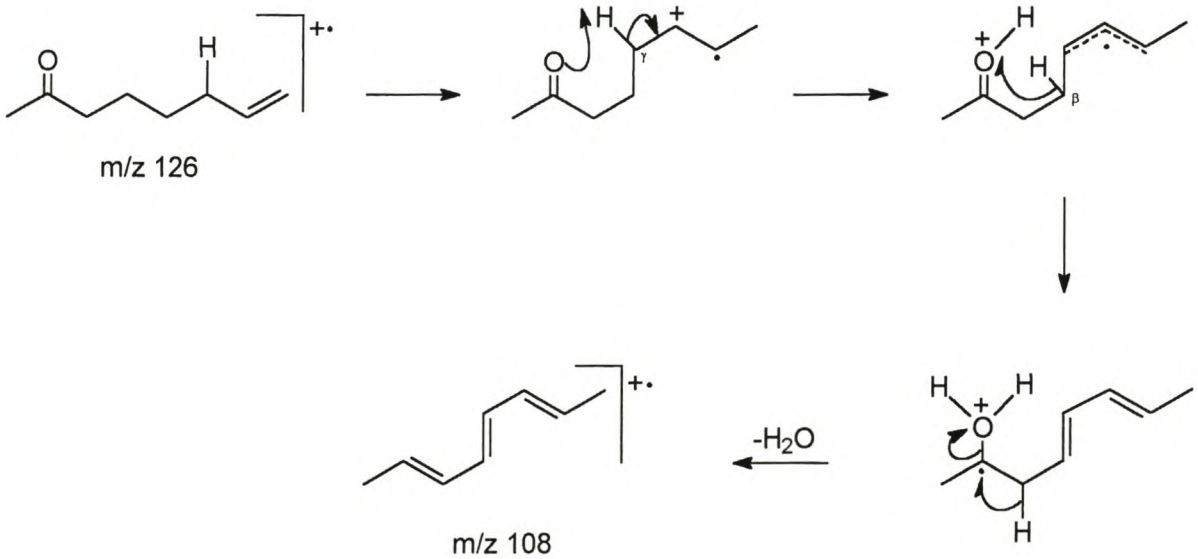
2.2.14 Ketones: Aliphatic (Unsaturated)

The EI mass spectrum of component 1452 (Fig. 2.50) has prominent ions at m/z 43 (base peak), 58, 68 (31%), 71, 85, 108, 111 and 126 (molecular ion). The ions at m/z 43 and m/z 58, the absence of an m/z 86 ion and the molecular ion at m/z 126 led to the conclusion that this could be an unsaturated methyl octenone. The prominent $[M-58]^{+\cdot}$ ion at m/z 68 is characteristic of olefinic ketones with at least four carbon atoms between the carbonyl group and the double bond[36]. Published data

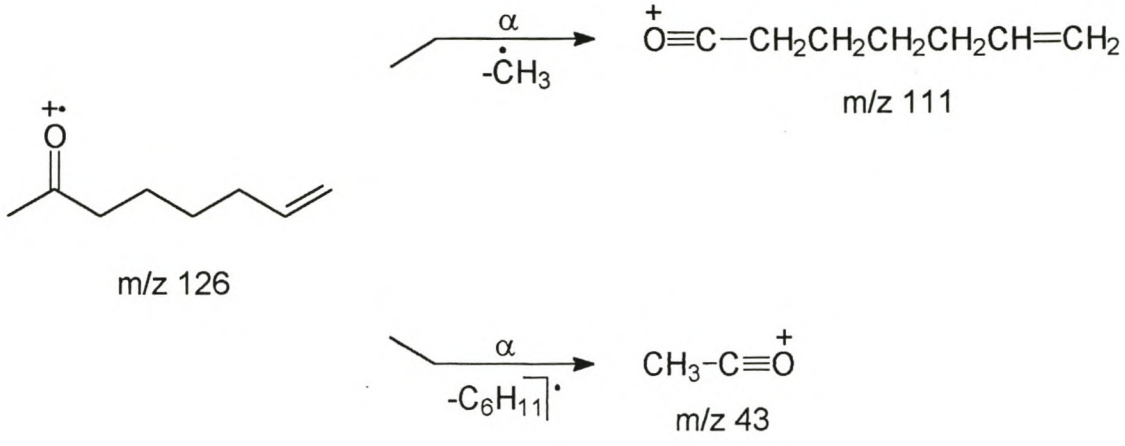
shows that the ion at m/z 68 is the base peak for 6-octen-2-one, while the relative intensity of this ion is less than 50% for 7-octen-2-one[37]. The working hypothesis that component 1452 could be 7-octen-2-one, was confirmed by co-injection of the commercially available ketone with the natural extract. The ion at m/z 68 in the mass spectrum of 7-octen-2-one can be rationalized as follows[37]:



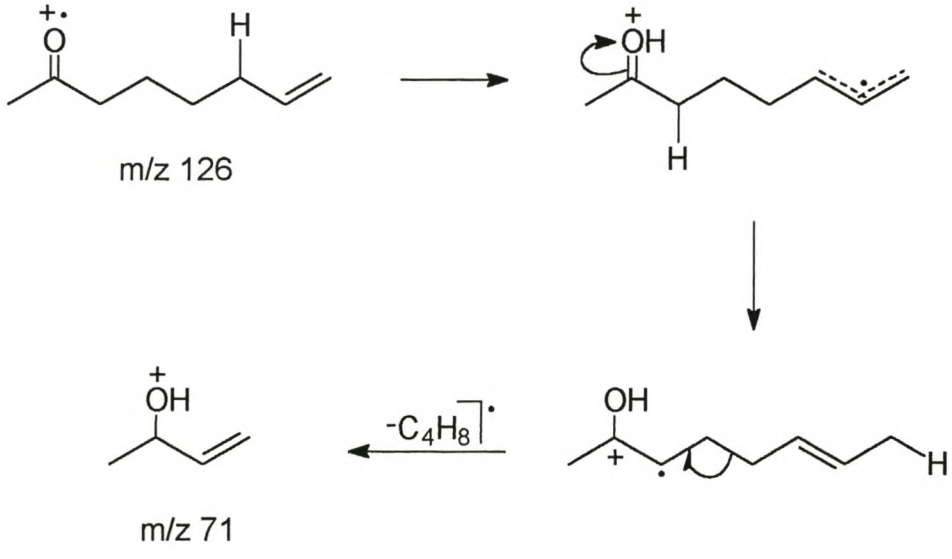
The ion at m/z 108 in this spectrum is formed through the elimination of water from the molecular ion. The main hydrogen atom donors for this process are those in the β- and γ-positions and a possible mechanism for the elimination of a water molecule can be formulated as follows:



The presence of a double bond does not influence the α-cleavage reactions[38] that produce the ions at m/z 43 and m/z 111:

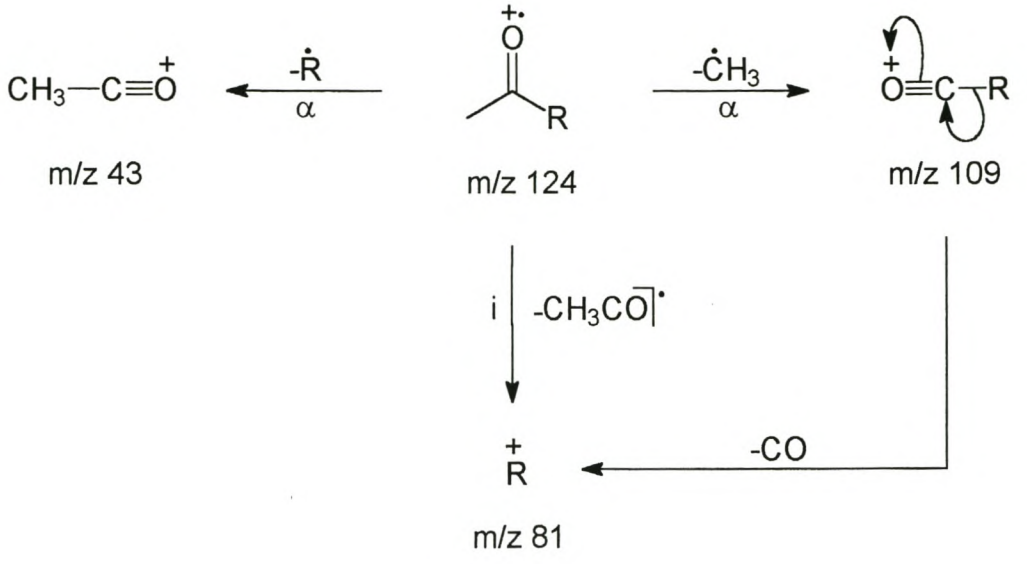


The fragment ion at m/z 71 is produced through the reciprocal hydrogen atom transfer involved in the fragmentation of saturated ketones[38]:

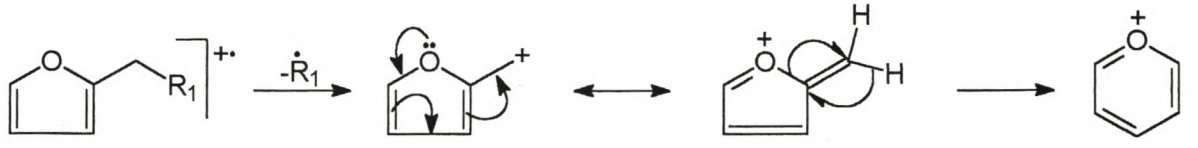


The only conclusions that could be drawn at first from the EI mass spectrum of component 2131 (Fig. 2.51), were the following:

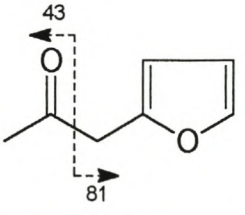
- The m/z 124 ion is the molecular ion.
- The m/z 109 ion (base peak) is an $[M\text{-}CH_3]^+$ ion.
- The m/z 81 ion (42%) could be an $[M\text{-}CH_3CO]^+$ and/or $[M\text{-}CH_3\text{-}CO]^+$ ion.
- The m/z 43 ion (15%) could be due to the presence of a methyl ketone moiety in the compound.



The R^+ ion at m/z 81 could either be $[C_6H_9]^+$ or $[C_5H_5O]^+$, the latter being formed by β-cleavage of alkylfurans[39]:

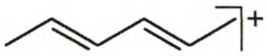


This would give 1-(2-furanyl)-2-propanone as a possibility:

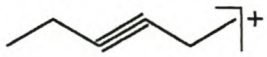


1-(2-Furanyl)-2-propanone

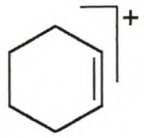
This compound is, however, expected to give rise to very abundant ions at m/z 43 $[CH_3CO]^+$ and m/z 81 $[C_5H_5O]^+$, and was therefore not considered. It was then assumed that the R^+ ion is $[C_6H_9]^+$, *i.e.*, formally one of the following:



dialkenyl



alkynyl

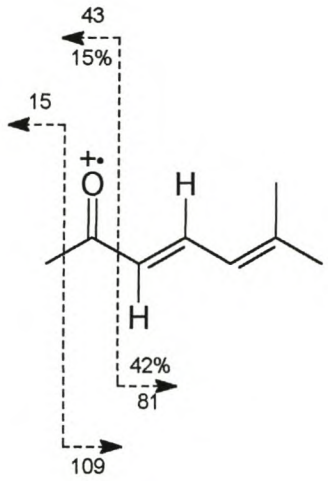


cycloalkenyl

A computer search using the following parameters was done[13]:

Molecular mass	:	124 Da
Molecular formula	:	$C_8H_{12}O$ (C_2H_3O + C_6H_9)
Name fragments	:	2-ketone & dialkene
Base peak	:	m/z 109

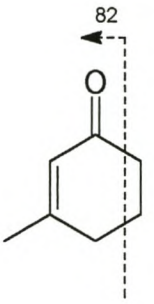
This yielded *trans*-6-methyl-3,5-heptadien-2-one as the only result and by co-injection of the synthetic *trans*-isomer with the natural extract, it was verified that component 2131 is *trans*-6-methyl-3,5-heptadien-2-one. The acylium ion formation, which requires vinylic cleavage to produce the m/z 43 ion, is suppressed, presumably because the alternative α-scission process, giving m/z 109, is favoured by the elimination of the small methyl radical:



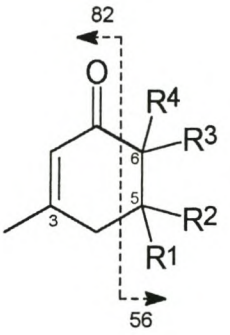
2.2.15 Ketones: Cyclic

The mass spectrum of component 2264 (Fig. 2.52) has a base peak at m/z 82 and a molecular ion at m/z 138 (22%). According to Budzikiewicz, Djerassi and

Williams[40], a number of cyclohexenones undergo a retro-Diels-Alder reaction to give a base peak at m/z 82 and a molecular ion of low relative abundance:



The loss of 56 atomic mass units from the molecular ion of component 2264 to give the m/z 82 base peak, is therefore due to the loss of a C_4H_8 olefin, giving the following three possible structures:

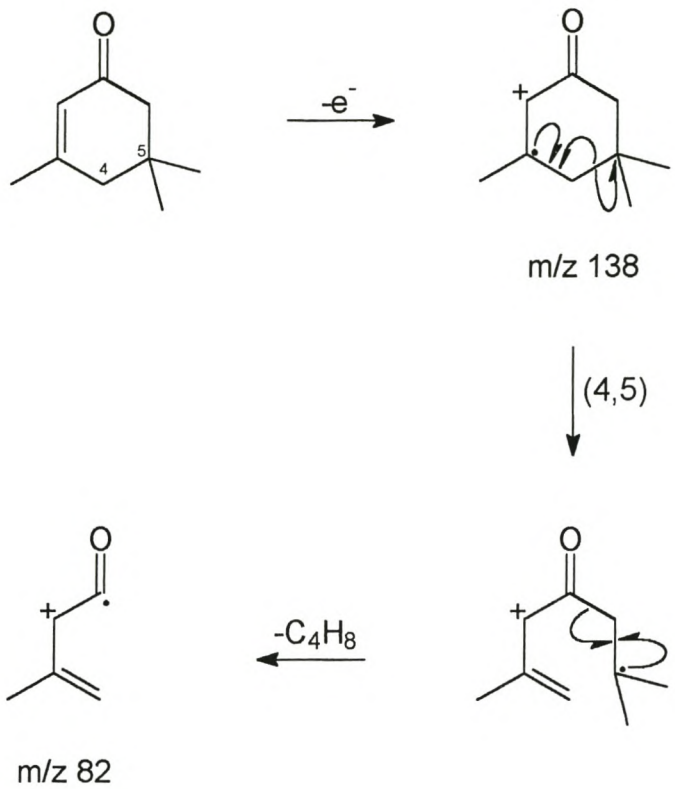


3,5,5-Trimethyl-2-cyclohexen-1-one ($R^1 = R^2 = CH_3$, $R^3 = R^4 = H$)
3,5,6-Trimethyl-2-cyclohexen-1-one ($R^1 = R^3 = CH_3$, $R^2 = R^4 = H$)
3,6,6-Trimethyl-2-cyclohexen-1-one ($R^1 = R^2 = H$, $R^3 = R^4 = CH_3$)

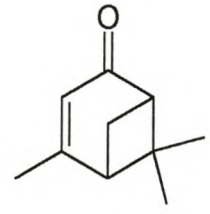
A computer search was done using the following parameters[13]:

Molecular mass	:	138 Da
Molecular formula	:	$C_9H_{14}O$
Base peak	:	m/z 82
Name fragments	:	cyclo & hexen & one & 3,x,y,trimethyl (x,y = 5,6)

This yielded 3,5,5-trimethyl-2-cyclohexen-1-one (isophorone) as the only possibility and co-injection of the commercially available α,β-unsaturated cyclic ketone with the natural extract proved that component 2264 is isophorone. The m/z 82 ion can be explained as follows:



A computerized library search[13] of the EI mass spectrum of component 2672 (Fig. 2.53) gave verbenone, also known as berbenone or 2-pinen-4-one, as a possibility:

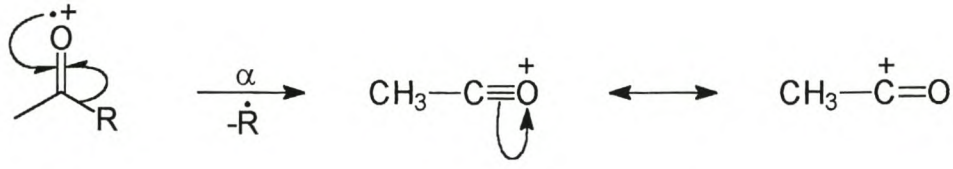


$C_{10}H_{14}O$, 150 Da

Co-injection of the natural extract with synthetic verbenone confirmed this identification.

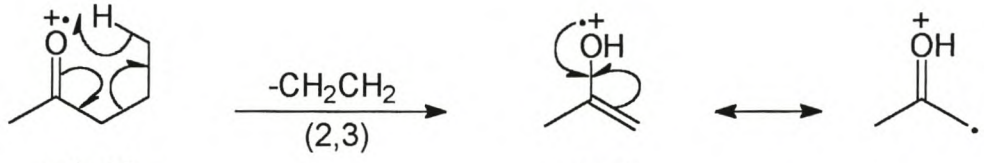
2.2.16 Diketones

The EI mass spectrum of component 238 (Fig. 2.54) has only two ions of interest, namely m/z 43 (base peak) and m/z 86 (molecular ion). If it is assumed that the m/z 43 ion is due to α-cleavage of a methyl ketone giving the acylium ion $[CH_3CO]^+$,



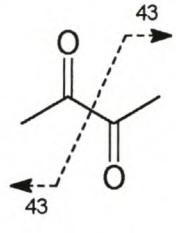
m/z 86 m/z 43

and that the loss of the 43 atomic mass units from the molecular ion at m/z 86 is due to the loss of either $[CH(CH_3)_2]^{\cdot}$, $[CH_2CH_2CH_3]^{\cdot}$ or $[CH_3CO]^{\cdot}$, component 238 could be 3-methyl-2-butanone, 2-pentanone or 2,3-butanedione (diacetyl). Component 314 has already been identified as 3-methyl-2-butanone (see § 2.2.13) and 2-pentanone has a McLafferty ion at m/z 58, which is not present in this mass spectrum:



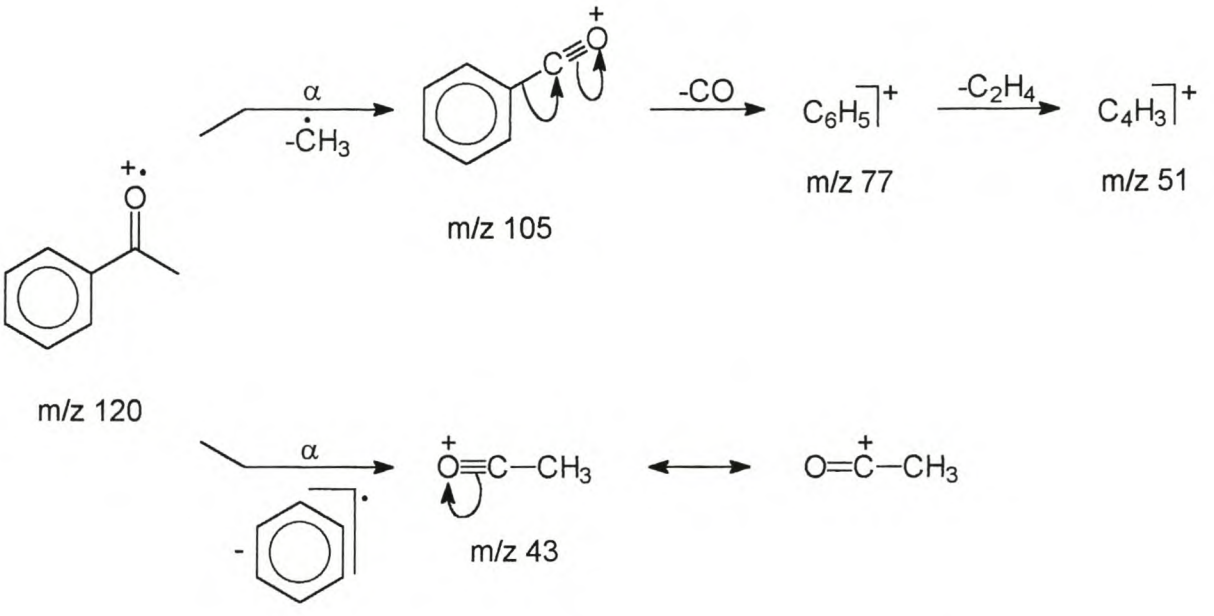
m/z 86 m/z 58

This led to the conclusion that component 238 could be 2,3-butanedione and co-injection of the natural extract with the synthetic compound confirmed this:

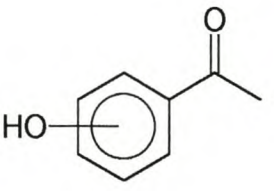


2.2.17 Ketones: Aromatic

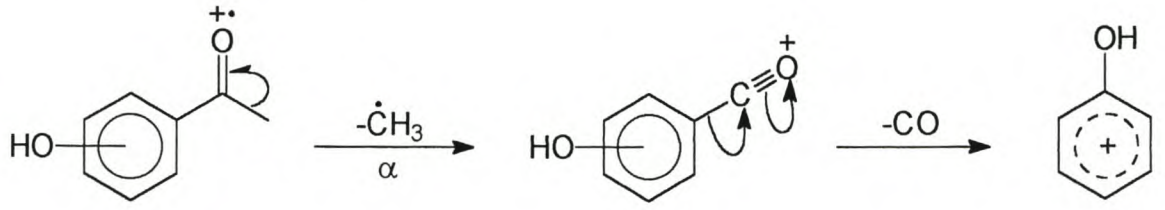
The EI mass spectrum of component 1939 (Fig. 2.55) is characteristic of acetophenone[41] and co-injection of the commercially available ketone with the natural extract confirmed the presence of this compound in the secretion. The prominent ions at m/z 120 (molecular ion), 105 (base peak), 77, 51 and 43 in the mass spectrum of this compound can be explained as follows[42]:



In the EI mass spectrum of component 4456 (Fig. 2.56), the base peak at m/z 121 corresponds to the loss of 15 atomic mass units from the ion at m/z 136. A further loss of 28 atomic mass units results in the ion at m/z 93. This fragmentation pattern, the loss of 15 followed by 28 atomic mass units, is characteristic of, amongst others, acetophenone and substituted acetophenones[43]. If the ion at m/z 136 is therefore assumed to be the molecular ion, it follows that a substituent having a mass of 17 Da, such as a hydroxyl group, must be present on the aromatic ring, giving the general structure shown below:

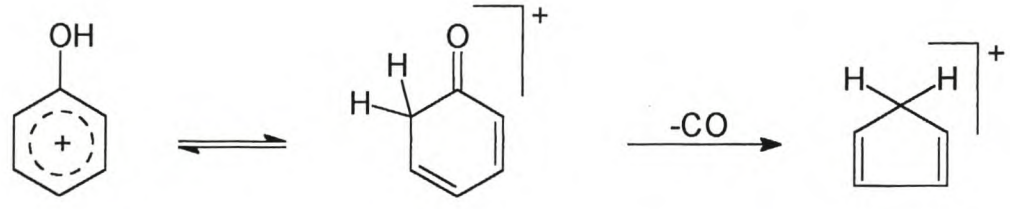


The loss of the methyl radical from the molecular ion represents the typical α-cleavage found in ketones, and this is followed by the subsequent elimination of CO to yield the ion at m/z 93. These processes can be illustrated as follows:



m/z 136 m/z 121 m/z 93

The only other ion of significant abundance in this spectrum is the one at m/z 65. The formation of this ion can be explained by the elimination of CO from the m/z 93 fragment. Based on the similarity of this species to phenol, this process can be formulated as follows:

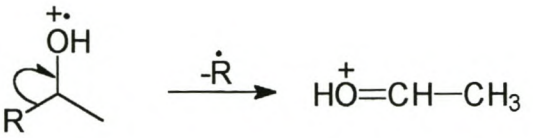


m/z 93 m/z 65

The position of the hydroxyl group on the aromatic ring could not be determined from the mass spectrum, but co-injection of the natural extract with commercially available 2- and 4-hydroxyacetophenone proved component 4456 to be the latter isomer.

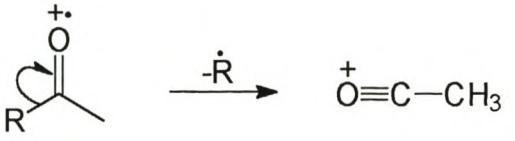
2.2.18 Hydroxy ketones: Aliphatic

The EI mass spectrum of component 536 (Fig. 2.57) has two very prominent ions at m/z 43 and m/z 45. An ion that could be the molecular ion appears at m/z 88, together with an $[M\text{-}CH_3]^+$ and $[M\text{-}CH_3\text{-}H_2O]^+$ ion at m/z 73 and m/z 55, respectively. The m/z 45 ion and the loss of a methyl radical and water suggest that this component could be a secondary methylcarbinol:



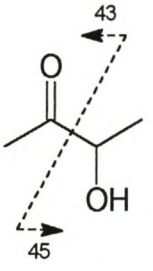
m/z 88 m/z 45

A very prominent m/z 43 ion is typical for methyl ketones:



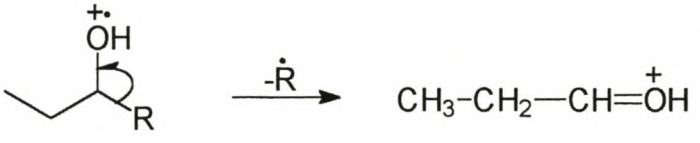
m/z 88 m/z 43

Since 43 + 45 = 88 Da, which was assumed to be the molecular mass of this component, 3-hydroxy-2-butanone (acetoin) was considered to be a likely candidate as α-cleavage can give the observed abundant ions at m/z 43 and m/z 45:

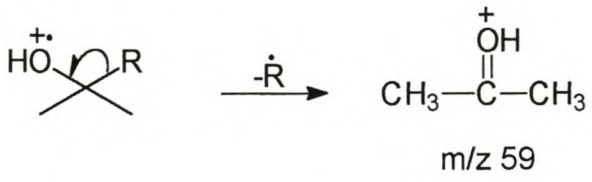


Co-injection of synthetic 3-hydroxy-2-butanone with the natural extract proved that component 536 is 3-hydroxy-2-butanone.

The base peak at m/z 59 in the EI mass spectrum of component 583 (Fig. 2.58) was at first attributed to a secondary methylcarbinol or tertiary alcohol, which can both give this ion as follows:



m/z 59



m/z 59

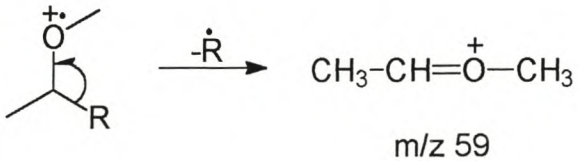
If the m/z 87 ion is taken as $[M\text{-}CH_3]^+$, the molecular formula of component 583 could be $C_6H_{14}O$. The following results were obtained with the respective isomeric hexanols:

Retention-time comparison of reference hexanols

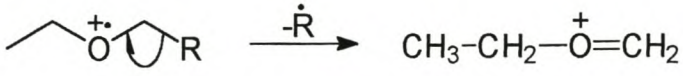
Alcohol	Source	Bp [°C]	RT	Other
2-Methyl-2-pentanol	C	120-122	Shorter	-
2,3-Dimethyl-2-butanol	S	120-121	Shorter	m/z 69 $[M\text{-}CH_3\text{-}H_2O]^+$
3-Hexanol	S	134-136	Longer	m/z 73 $[M\text{-}C_2H_5]^+$
2-Methyl-3-pentanol	C	128	Shorter	m/z 73 $[M\text{-}C_2H_5]^+$

C = Commercial, S = Synthetic, RT = Retention time

Other options that were also investigated as possible candidate compounds, were the 2-methoxyalkanes and 1-ethoxyalkanes (ethyl ethers):



m/z 59



m/z 59

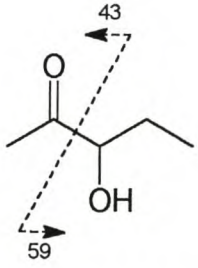
The following results were obtained and deduced for these ethers:

Retention-time comparison of reference ethers

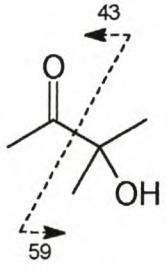
Ether	Source	Bp [°C]	RT	Other
Methyl 2-pentyl ether	S	~80	Shorter	Weak m/z 43 ion
Methyl 3-methyl-2-butyl ether	-	<80	Shorter	RT shorter than methyl 2-pentyl ether because of branching
Butyl ethyl ether	C	91-92	Shorter	M^+ at m/z 102
Ethyl isobutyl ether	-	<92	Shorter	RT shorter than butyl ethyl ether because of branching
sec-Butyl ethyl ether	-	<92	Shorter	Base peak at m/z 45
tert-Butyl ethyl ether	-	72-73	Shorter	Weak m/z 43 and strong m/z 87 ions

C = Commercial, S = Synthetic, RT = Retention time

The next step was to assume, as was done in the case of component 536 (3-hydroxy-2-butanone), that the m/z 43 ion has to be taken as evidence in favour of the compound being a methyl ketone. If the molecular mass is assumed to be 102 Da (= 43 + 59), the following two possibilities arose:

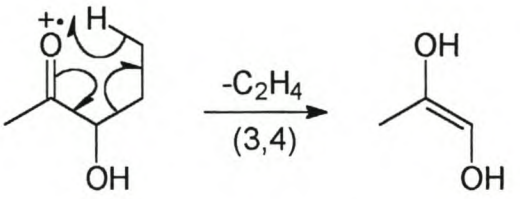


3-Hydroxy-2-pentanone



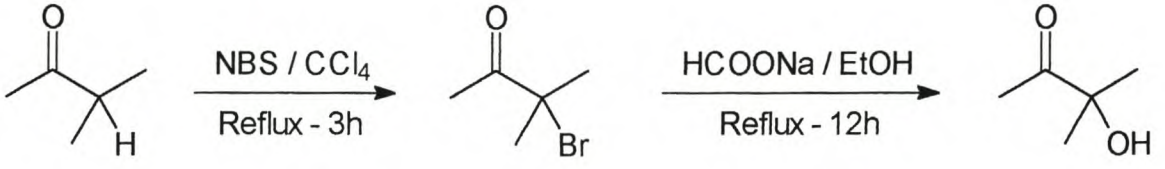
3-Hydroxy-3-methyl-2-butanone

The mass spectrum of 3-hydroxy-2-pentanone has a McLafferty rearrangement ion at m/z 74,



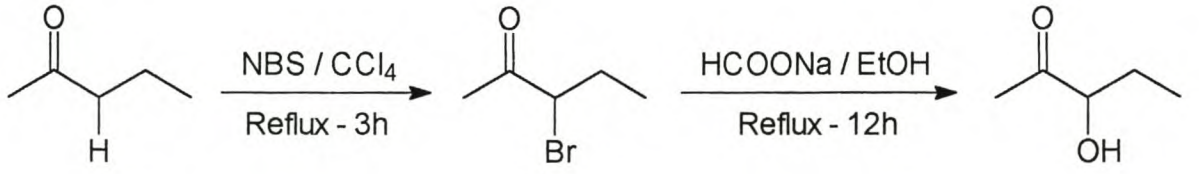
m/z 102 m/z 74

and therefore 3-hydroxy-3-methyl-2-butanone was synthesized according to the scheme shown below as first candidate:

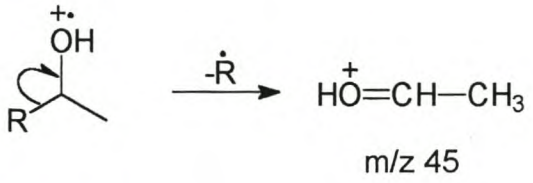


The mass spectrum of the synthetic 3-hydroxy-3-methyl-2-butanone [see § 3.4.10 and mass spectrum Fig. 3.10(c)] was compared to the mass spectrum of component 583 and found to be almost identical, as was the retention times of the synthetic product and the natural extract. Co-injection of the synthetic hydroxy ketone and the natural extract proved that component 583 is 3-hydroxy-3-methyl-2-butanone.

Investigation of the EI mass spectrum of component 805 (Fig. 2.59) in the TIC revealed similarities between this mass spectrum and the mass spectrum of 3-hydroxy-3-methyl-2-butanone (component 583, Fig. 2.58), discussed previously. Component 805 also displays an m/z 74 ion, which could be attributed to a McLafferty rearrangement in 3-hydroxy-2-pentanone, as stated before. This compound was synthesized according to the scheme shown below [see § 3.4.11 and mass spectrum Fig. 3.11(c)] and co-injection of the synthetic product with the natural interdigital extract proved that component 805 is 3-hydroxy-2-pentanone.

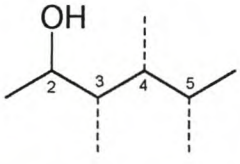


The m/z 45 ion in the EI mass spectrum of component 842 (Fig. 2.60) could be as a result of the following mechanism:



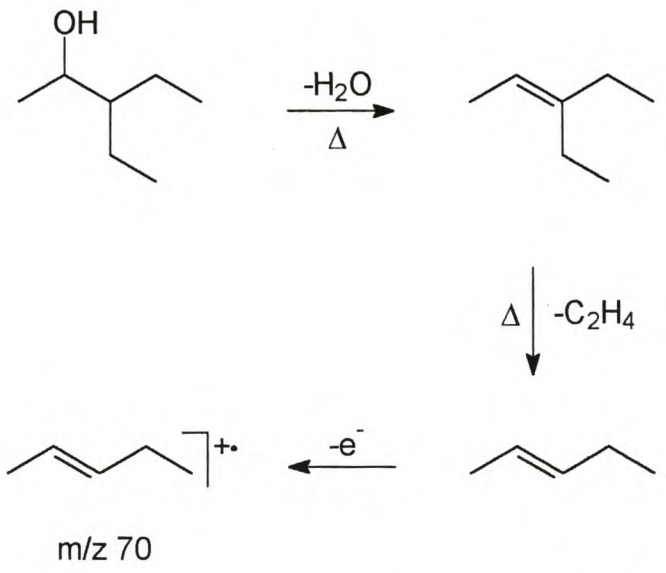
m/z 45

Close inspection of the mass spectrum showed a very weak ion at m/z 102 (3%) and one at m/z 87 (4%), which could be an $[M\text{-}CH_3]^+$ ion. If the ion at m/z 102 is assumed to be the molecular ion and the m/z 45 ion is taken to be the result of an α-cleavage of a 2-alkanol, as was shown above, component 842 must have a molecular formula of $C_6H_{12}O$. The injection of commercially available 2-hexanol for retention-time testing, showed that its retention time was too short. This meant that the hexanol isomers, 3- and 4-methyl-2-pentanol, would have retention times too short to be component 842, because retention time decreases with the introduction of branching. Commercially available 2-heptanol was tested next; this time the retention time was too long. Commercially available 3-methyl-2-hexanol also gave a retention time that was too long, which meant that the retention times of the remaining heptanol isomers, 4- and 5-methyl-2-hexanol, would also be too long, because retention time increases as the methyl group is moved away from the carbon atom carrying the hydroxyl group:



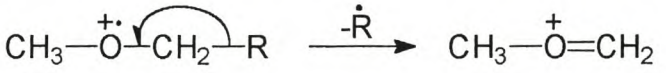
Retention time: 5 > 4 > 3

3-Ethyl-2-pentanol, another heptanol isomer, has an abundant m/z 70 ion, which has been explained[7] in terms of the thermal 1,2-elimination of water and ethylene prior to ionization:



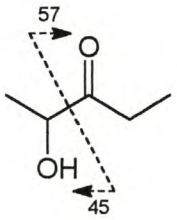
m/z 70

This ruled out 3-ethyl-2-pentanol as a candidate. Methyl ethers are another group of compounds giving m/z 45 ions as follows:

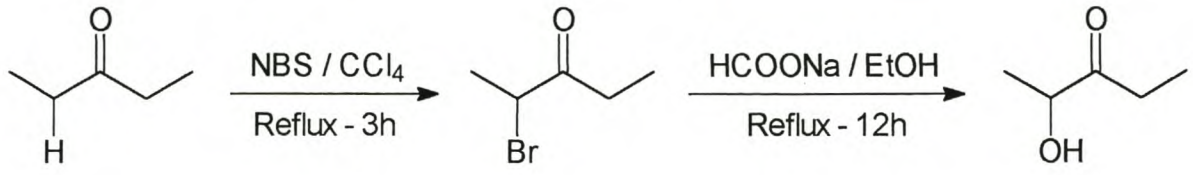


m/z 45

As was shown for component 583, however, ethers have much shorter retention times than alcohols of the same molecular formula, and they were therefore not considered. It was then hypothesized that component 842 could have a molecular formula of $C_5H_{10}O_2$. In the investigation of the previous two hydroxy ketones, components 536 and 583, respectively, it was noted that there were only two prominent, characteristic ions in each mass spectrum and that the sum of these two ions gave the molecular ion (43 + 45 = 88 Da and 43 + 59 = 102 Da). This is also true for component 842, where 45 + 57 = 102 Da. There cannot, however, be a methyl ketone group in the compound, because of the weak m/z 43 ion. That left the compound shown below, namely 2-hydroxy-3-pentanone, as a possible candidate:



2-Hydroxy-3-pentanone was synthesized according to the scheme shown below [see § 3.4.12 and mass spectrum Fig. 12(b)] and co-injection of the synthetic product with the natural extract showed that component 842 is 2-hydroxy-3-pentanone.

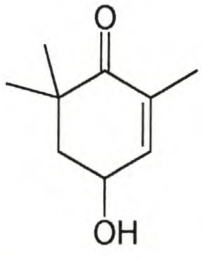


2.2.19 Hydroxy ketones: Cyclic

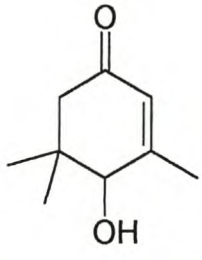
Although the EI mass spectrum of component 3105 (Fig. 2.61) has prominent ions, for example m/z 69, 70, 98 (base peak), 111 and 154 (molecular ion), no useful information could initially be extracted from it. A computer search using the following parameters was therefore done[13]:

Molecular mass	:	154 Da
Base peak	:	m/z 98
Relative abundance	:	m/z 154, 2-18%
		m/z 70, 44-66%

The following two compounds were returned as possible candidates:



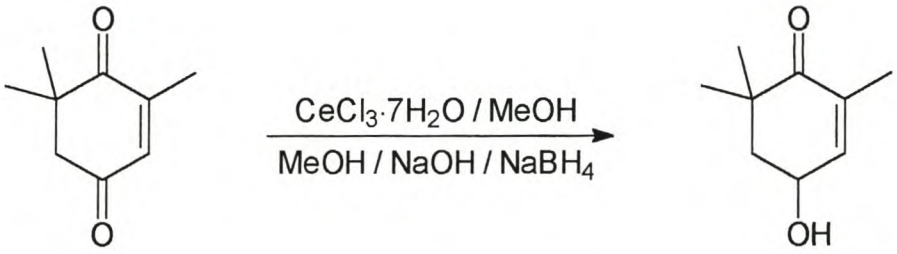
4-Hydroxy-2,6,6-trimethyl-
2-cyclohexen-1-one



4-Hydroxy-3,5,5-trimethyl-
2-cyclohexen-1-one

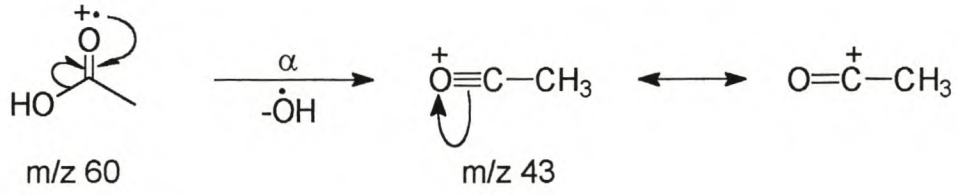
McLafferty and Turecek propose $[C_6H_{10}O]^{+\cdot}$ as a possible formula for an m/z 98 ion and give 2-alkylcycloalkanone as a possible parent compound type[44]. They also give cyclic ketones as possible compounds that can lose a neutral fragment of 56 atomic

mass units (C_2H_4CO, 154 – 56 = 98 Da)[45]. These two factors, together with the results of the computer search using the Wiley library, prompted the synthesis of 4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one according to the scheme shown below [see § 3.4.13 and mass spectrum Fig. 3.13(a)], and co-injection of the synthetic product with the natural secretion of the black wildebeest, confirmed that component 3105 is 4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one.

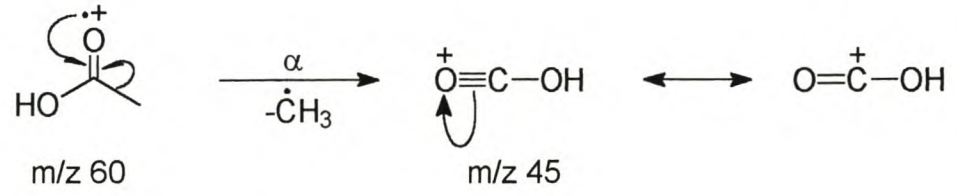


2.2.20 Carboxylic acids: Aliphatic (Saturated)

The EI mass spectrum of component 367 (Fig. 2.62) has prominent ions at m/z 43 (base peak), 45 and 60 (molecular ion). This is characteristic for acetic acid and co-injection of the interdigital secretion with the commercially available acid confirmed that component 367 is in fact acetic acid. The ion at m/z 43 is formed by a simple α-cleavage reaction:

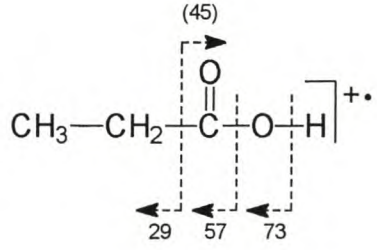


In the case of the ion at m/z 45, the formation of a $[COOH]^+$ fragment seems obvious[46]:



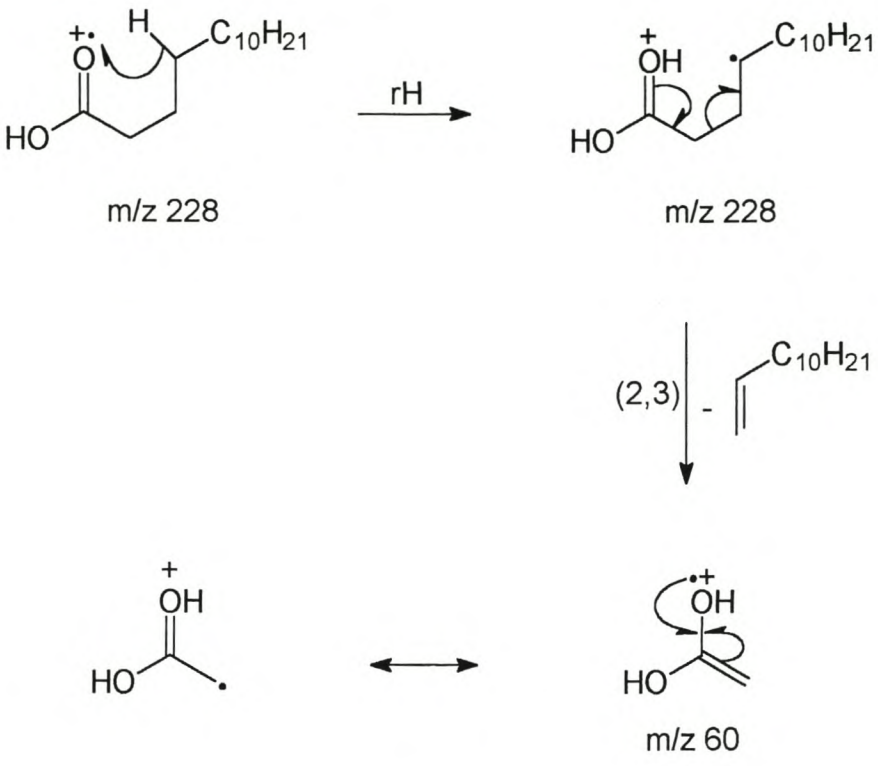
However, the ion at m/z 45 has been shown to have the structure $[OC_2H_5]^+$, but the mechanism of formation of this species is not known[47].

The EI mass spectrum of component 667 (Fig. 2.63) exhibits two prominent ions at m/z 74 and m/z 73. These ions are characteristic for propanoic acid[48] and represent the molecular ion and the $[M-1]^+$ ion, respectively, which is formed by elimination of the acid proton. The ion at m/z 57 could be attributed to loss of a hydroxyl group by α-cleavage. These simple cleavage reactions are illustrated as follows:

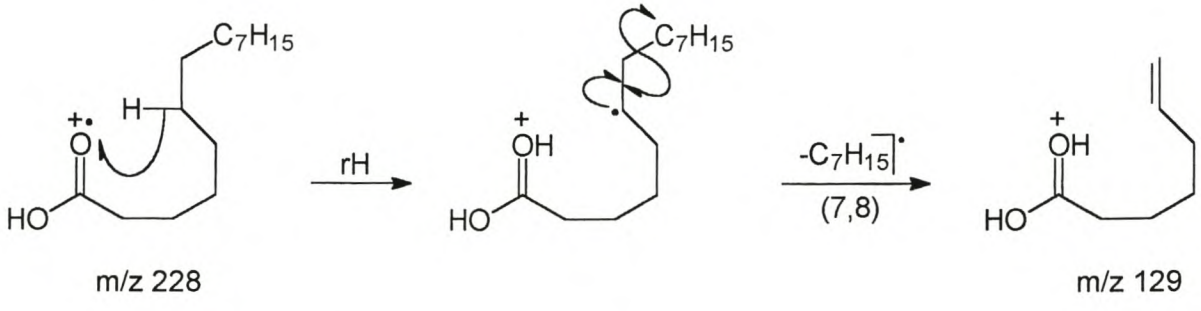


Co-injection of the natural extract with the synthetic acid confirmed component 667 to be propanoic acid.

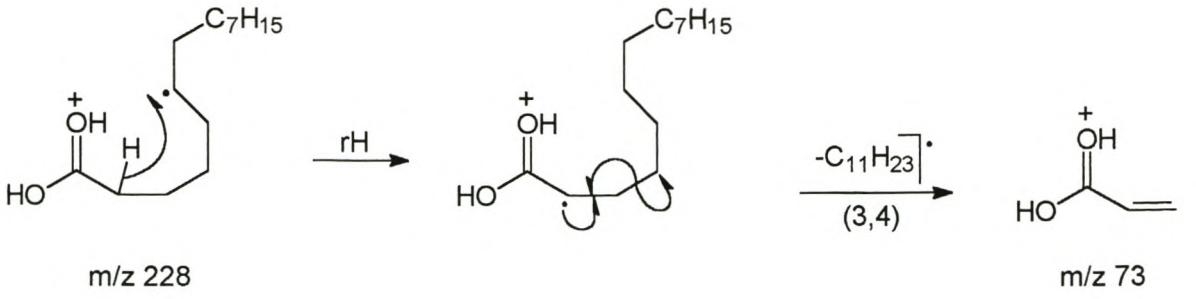
The EI mass spectra of components 957 (Fig. 2.64), 1375 (Fig. 2.65), 1807 (Fig. 2.66), 3052 (Fig. 2.67), 3432 (Fig. 2.68), 4154 (Fig. 2.69), 4494 (Fig. 2.70), 4825 (Fig. 2.71), 5137 (Fig. 2.72), 5458 (Fig. 2.73), 5736 (Fig. 2.74) and 6028 (Fig. 2.75) are characterized by the presence of a prominent ion at m/z 60, indicating that all these components are aliphatic acids[49], probably belonging to the same homologous series. The EI mass spectrum of component 4825 will be discussed as representative of this series of compounds. Acids generally have reasonably prominent molecular ions, the relative abundance of which increase with molecular mass for unbranched aliphatic acids containing more than six carbon atoms[50]. For this reason, the ion at m/z 228 in the mass spectrum of component 4825 was assumed to be the molecular ion and this component was therefore presumed to be tetradecanoic acid ($C_{14}H_{28}O_2$, 228 Da). The ion at m/z 60 is formed by the following McLafferty rearrangement[51]:



A prominent series of ions in this EI mass spectrum occurs at m/z 73, 115, 129, 143, 157, *etc.* These ions are formed by hydrogen transfer from carbon atoms along the carbon chain of the acid, in conjunction with homolysis of one of the bonds β to these hydrogen-depleted carbon atoms. In the case of the m/z 129 ion, the process can be illustrated as follows[52]:

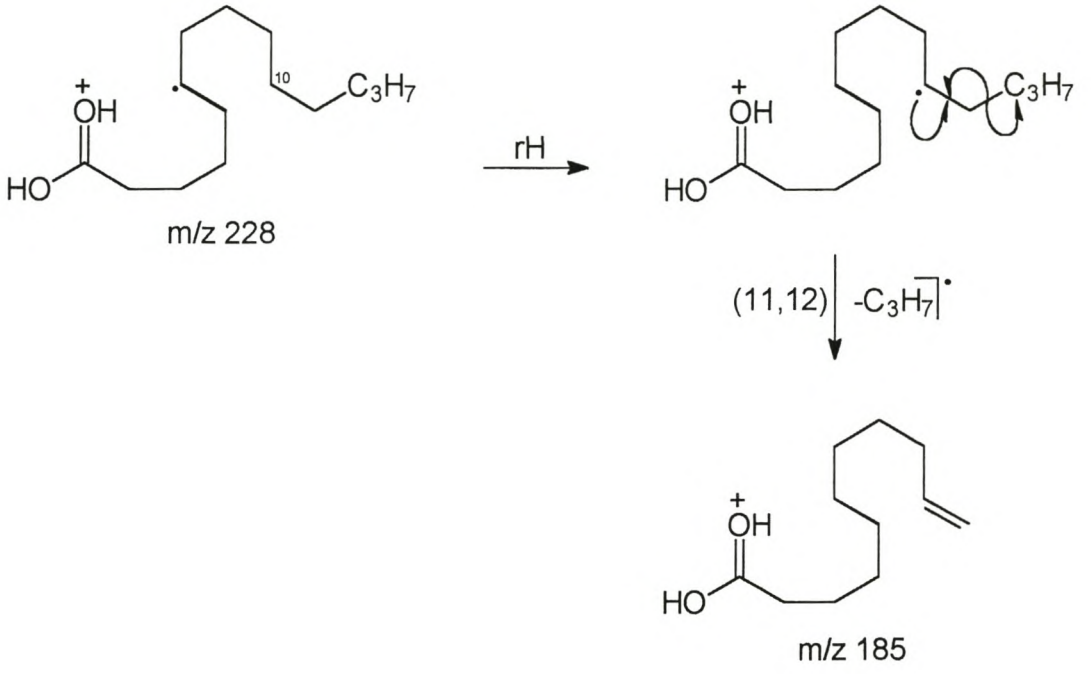


The most favoured of these reactions is the one resulting in the ion at m/z 73, formed by the transfer of a hydrogen atom from the α-position (relative to the carbonyl group) and subsequent β-cleavage. The driving force for this reaction is the high stability of the α,β-unsaturated protonated carbonyl system. The resulting [McLafferty + 13] ion often accompanies the normal McLafferty rearrangement ion[53]:



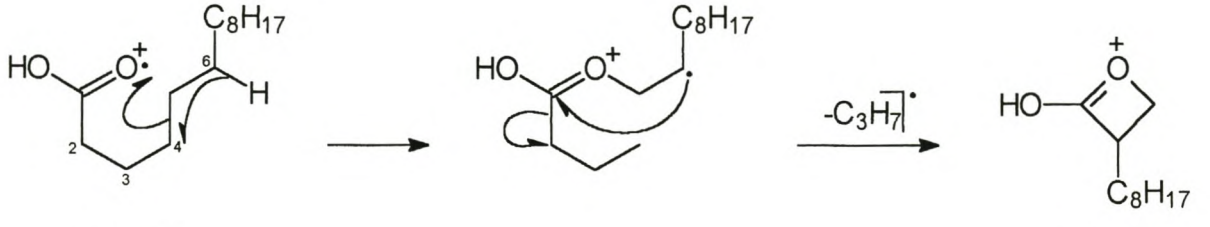
m/z 228 m/z 73

In the same manner, hydrogen migration from C10, followed by β-cleavage, results in the ion at m/z 185:



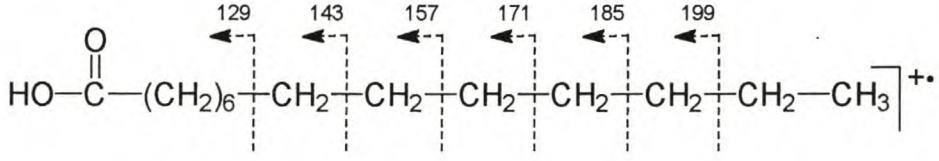
m/z 228 m/z 185

The appearance of an ion at m/z 185 also corresponds to the loss of 43 atomic mass units from the molecular ion, and can be explained by a rearrangement reaction involving the transfer of a hydrogen atom from C6, resulting in the elimination of the fragment containing carbon atoms C2-C4:



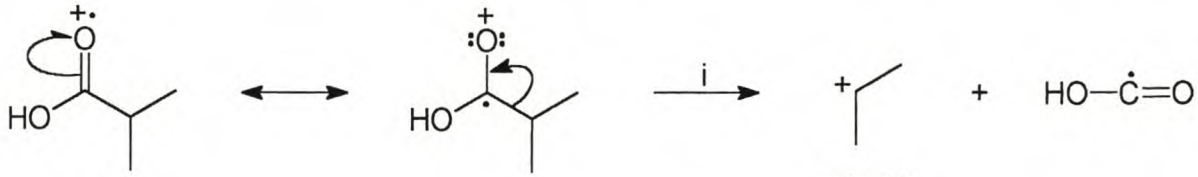
m/z 228 m/z 185

The formation of this series of ions at m/z 199 $[M-29]^+$, 185 $[M-43]^+$, 171 $[M-57]^+$, 157 $[M-71]^+$, *etc.*, could also be explained in terms of simple α-cleavage with the charge being retained on the oxygen-containing fragments. α-Cleavage with charge retention on the alkyl groups accounts for many of the ions in the lower mass range of the spectrum:



Co-injection of the extract of the natural secretion with a series of synthetic acids (butanoic acid through docosanoic acid) confirmed that components 957, 1375, 1807, 3052, 3432, 4154, 4494, 4825, 5137, 5458, 5736 and 6028 are butanoic, pentanoic, hexanoic, nonanoic, decanoic, dodecanoic, tetradecanoic, pentadecanoic, hexadecanoic, heptadecanoic and octadecanoic acid, respectively. It is not possible to deduce from the mass spectra of especially the long chain acids whether the acids are branched or unbranched, and it was therefore necessary to co-inject the secretion with unbranched and methyl-branched aliphatic acids. The retention time of a branched acid is shorter than that of the corresponding unbranched acid.

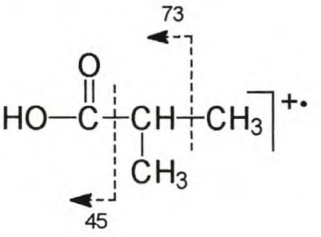
Component 829 (Fig. 2.76) exhibits a base peak at m/z 43 and other prominent ions at m/z 45, 60 (3%), 73 and 88 (molecular ion), which are indicative of saturated, aliphatic acids. The molecular ion at m/z 88 indicates that it is a butanoic acid, but the absence of a high-abundance m/z 60 McLafferty ion excludes butanoic acid. The only branched structural isomer of butanoic acid, 2-methyl propanoic acid, was co-injected with the natural extract and found to have the same retention time as component 829, which is shorter than that of butanoic acid, component 957. The base peak at m/z 43 is formed through a charge site initiation (inductive cleavage):



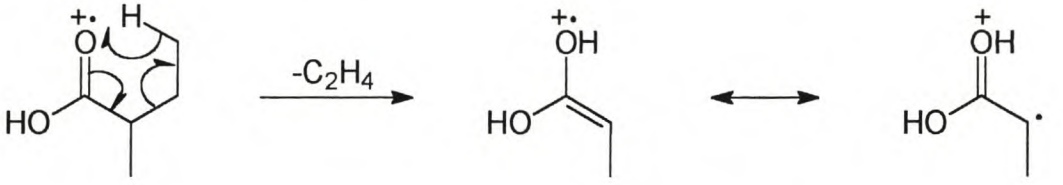
m/z 88

m/z 43

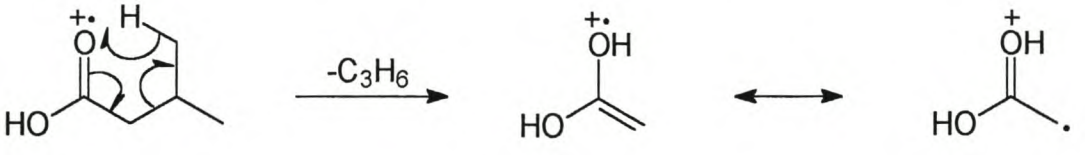
The m/z 45 and m/z 73 ions are formed through the following α-cleavages:



The EI mass spectrum of component 1181 (Fig. 2.77) has a base peak at m/z 60, with ions at m/z 87, 45 and 43 of lesser abundance. The m/z 43, 45 and 60 ions indicate that component 1181 could be a saturated, aliphatic acid. The molecular ion is not visible, but m/z 87 could be an $[M-15]^+$ ion, which would indicate a molecular ion at m/z 102. Component 1181 could therefore be a pentanoic acid, but the shorter retention time than that of pentanoic acid (component 1375) indicates the presence of branching. 2-Methylbutanoic acid and 3-methylbutanoic acid are the only two possible branched pentanoic acid isomers. Each of these two acids undergoes a McLafferty rearrangement, but 2-methylbutanoic acid gives an m/z 74 McLafferty ion compared to the m/z 60 McLafferty ion of 3-methylbutanoic acid:



m/z 102 m/z 74

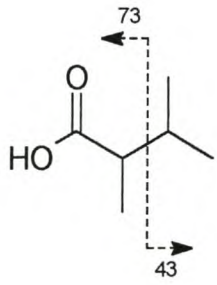


m/z 102 m/z 60

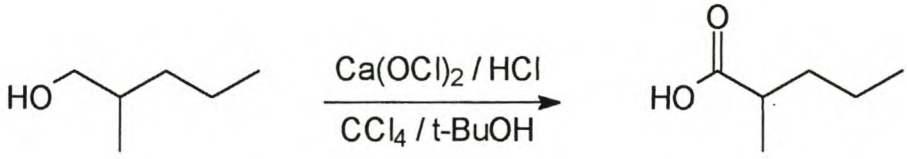
The EI mass spectrum of component 1206 (Fig. 2.78) has a base peak at m/z 74 and a retention time 0.5 minutes longer than that of component 1181. It was therefore assumed that this component could be 2-methylbutanoic acid. Co-injection of the

natural secretion with a mixture of synthetic 2- and 3-methylbutanoic acid confirmed that components 1181 and 1206 are 3- and 2-methylbutanoic acid, respectively.

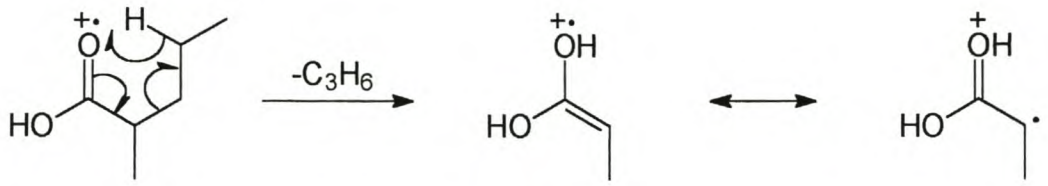
Component 1588 (Fig. 2.79) has a base peak at m/z 74, and as was seen in the case of 2-methylbutanoic acid (component 1206), this was an indication of an α-methyl acid. Since component 1588 has a retention time shorter than hexanoic acid (component 1807) but longer than pentanoic acid (component 1375), and since the difference in retention time between 2-methylbutanoic acid and 2-methylpropanoic acid (component 829) is 6.3 minutes and the difference in retention time between component 1588 and 2-methylbutanoic acid is 6.4 minutes, it was assumed that component 1588 could be 2-methylpentanoic acid. 2,3-Dimethylbutanoic acid was not considered because of the absence of an m/z 87 $[M\text{-}CH_2CH_3]^+$ ion in the mass spectrum of this dimethyl acid:



2-Methylpentanoic acid was synthesized by oxidation of 2-methyl-1-pentanol with calcium hypochlorite according to the scheme shown below (see § 3.4.14 and mass spectrum Fig. 3.14) and co-injection of the synthetic product with the natural extract proved that component 1588 is 2-methylpentanoic acid.

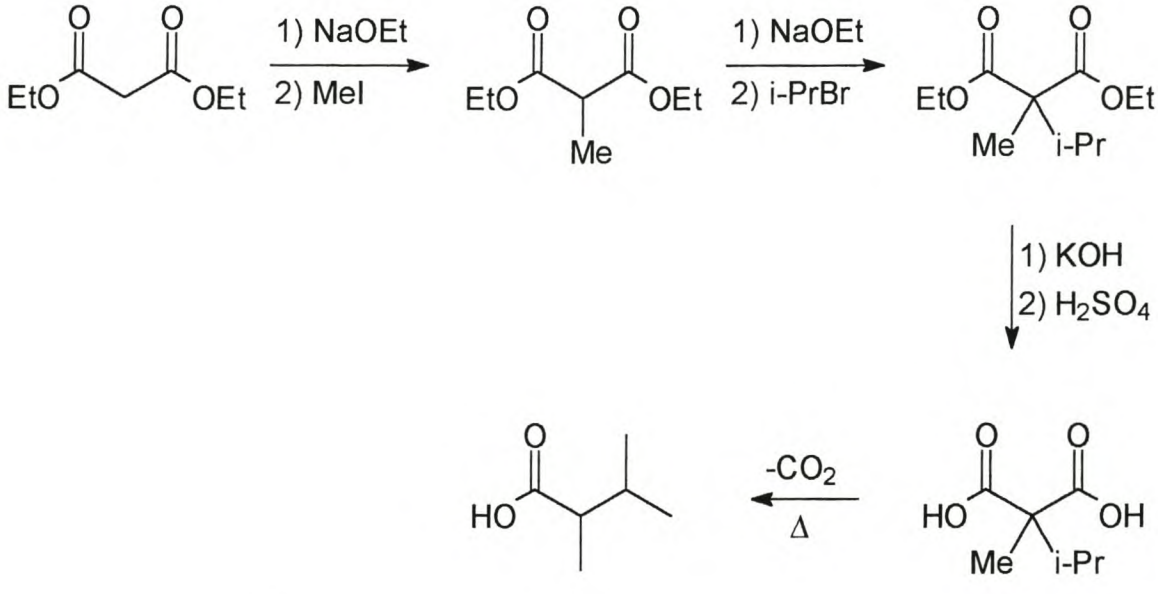


The McLafferty rearrangement giving the m/z 74 ion can be formulated as follows:

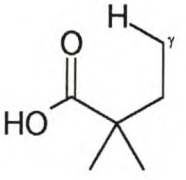


m/z 116 m/z 74

The EI mass spectrum of component 1510 (Fig. 2.80) is almost exactly identical to that of 2-methylpentanoic acid, component 1588; the only major differences being the presence of ions at m/z 101 and m/z 83, and the absence of an $[M\text{-}CH_2CH_3]^+$ ion at m/z 87 in the mass spectrum of component 1510. If the ions at m/z 101 and m/z 83 are the $[M\text{-}CH_3]^+$ and $[M\text{-}H_2O\text{-}CH_3]^+$ ions, respectively, and if the m/z 74 ion (base peak) again indicates an acid with a methyl group on C2, component 1510 could be a 2-methylhexanoic acid. Since component 1588 has already been identified as 2-methylpentanoic acid and the retention time of component 1510 is 78 seconds shorter than that of component 1588, component 1510 could only be 2,3-dimethylbutanoic acid. This acid was synthesized according to the following reaction scheme (see § 3.4.15 and mass spectrum Fig. 3.15) and co-injected with the natural interdigital extract, to prove that component 1510 is indeed 2,3-dimethylbutanoic acid.

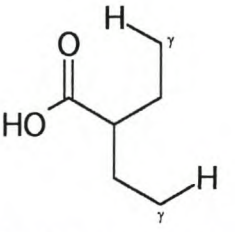


The EI mass spectrum of component 1559 (Fig. 2.81) displays three prominent ions at m/z 43, 73 and 88, all having relative abundance of more than 85%, and ions of less than 50% relative abundance at m/z 45, 55, 60, 87 and 101. The m/z 45 and m/z 60 ions have already been shown to be characteristic of aliphatic acids and if it is assumed that m/z 87 is the $[M\text{-}C_2H_5]^+$ ion and m/z 101 the $[M\text{-}CH_3]^+$ ion, component 1559 could be a hexanoic acid. The absence of an abundant m/z 60 McLafferty rearrangement ion and the presence of an abundant m/z 88 ion indicates branching at C2, but the presence of the m/z 60 ion, however small, suggests that a second McLafferty does take place. This leaves 2-ethylbutanoic acid as the only possible candidate structure:



2,2-Dimethylbutanoic acid

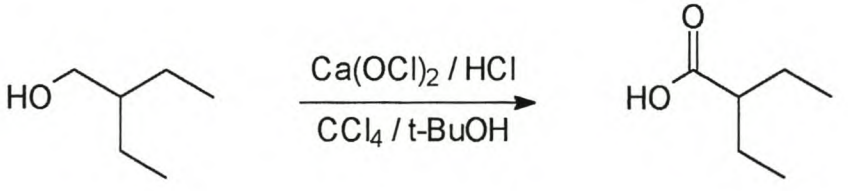
One McLafferty rearrangement possible, therefore only m/z 88 is observed and no m/z 60.



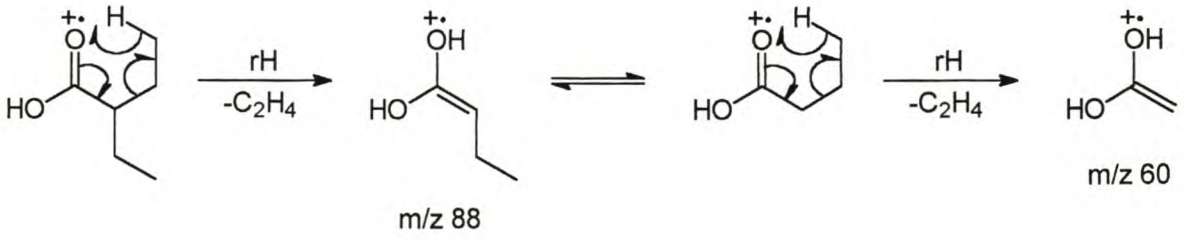
2-Ethylbutanoic acid

Two McLafferty rearrangements possible, therefore both m/z 88 and m/z 60 are observed.

2-Ethylbutanoic acid was synthesized according to the scheme shown below (see § 3.4.16 and mass spectrum Fig. 3.16) and the product was co-injected with the natural extract, to confirm that component 1559 is 2-ethylbutanoic acid.



Some of the ions under discussion are formed as follows:

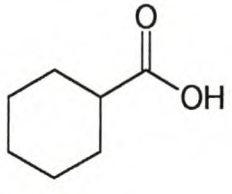


2.2.21 Carboxylic acids: Aliphatic (Unsaturated)

The EI mass spectrum of component 5986 (Fig. 2.82) has an ion at m/z 60 with a relative abundance lower than that of a typical saturated aliphatic acid. In addition, it contains a prominent series of ions at m/z 41, 55, 69, 83, 97, *etc.* corresponding to the general formula $[C_nH_{2n-1}]^+$, which is characteristic of unsaturation in aliphatic acids. This component has a retention time 0.7 minutes shorter than that of octadecanoic acid, component 6028, which could indicate unsaturation or branching. The m/z 264 ion was assumed to be an $[M-H_2O]^+$ ion, which would require a molecular mass of 282 Da. Component 5986 could therefore be an octadecenoic acid. A computer search done on both the NBS and Wiley libraries[13] confirmed this assumption by returning (*Z*)-9-octadecenoic acid as a likely candidate structure. Because (*Z*)-9-octadecenoic acid had already been found in many mammalian exocrine secretions[54], it was considered a likely candidate, and by co-injection of the synthetic acid with the natural secretion, component 5986 was indeed found to be (*Z*)-9-octadecenoic acid.

2.2.22 Carboxylic acids: Cyclic

Component 2486 (Fig. 2.81) was identified as cyclohexanecarboxylic acid, the structure of which is shown below, with the aid of published mass spectra[13]. Confirmation of the presence of this compound in the secretion was obtained as usual by co-injection of the commercially available acid with the natural extract.



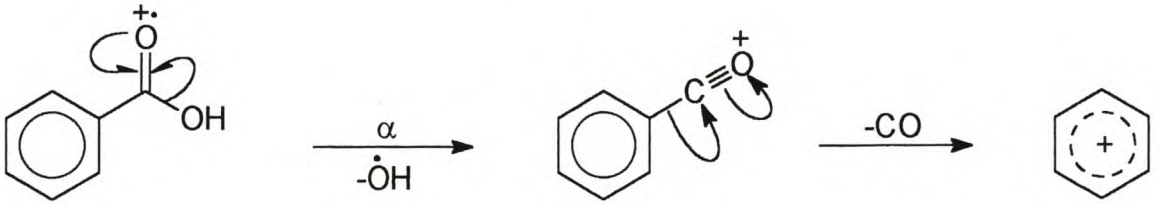
Although no relevant high resolution data are available, some of the ions in the mass spectrum of this compound were assumed to be formed by the expulsion from the molecular ion of the fragments shown in the following table.

Formation of ions in the EI mass spectrum of cyclohexanecarboxylic acid, component 2486.

m/z	Ion
128	$M^{+\cdot}$
113	$[M-CH_3]^+$
110	$[M-H_2O]^{+\cdot}$
99	$[M-C_2H_5]^+$
83	$[M-COOH]^+$ or $[M-OH-CO]^+$
73	$[M-C_4H_7]^+$
68	$[M-C_3H_7OH]^+$ or $[M-CO-CH_3OH]^+$
55	$[M-COOH-C_2H_4]^+$

2.2.23 Carboxylic acids: Aromatic

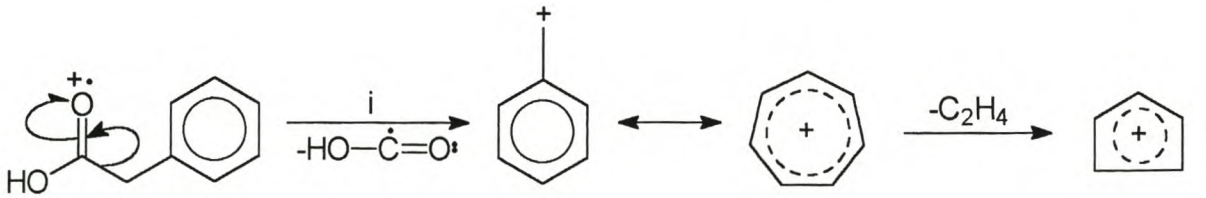
The EI mass spectrum of component 2912 (Fig. 2.84) shows a very good correlation with data published for benzoic acid. This was confirmed by gas chromatographic co-elution of the natural compound and synthetic benzoic acid. The formulation of the ions at m/z 77, 105 (base peak) and 122 (molecular ion) in the mass spectrum of this compound can be explained as follows[55]:



m/z 122 m/z 105 m/z 77

The EI mass spectrum of component 3260 (Fig. 2.85) has its base peak at m/z 91, which, in aromatic systems, is attributed to the formation of the tropylium ion $[C_7H_7]^+$. Since the molecular ion is usually more abundant in aromatic compounds,

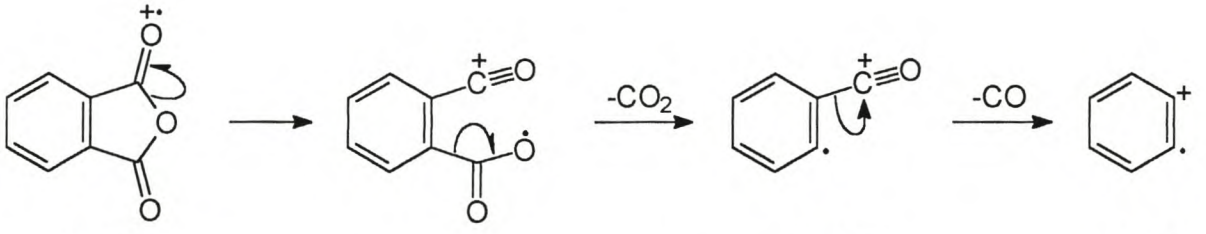
the ion at m/z 136 was assumed to be the molecular ion. The difference between m/z 136 and m/z 91 is 45 atomic mass units, which could be ascribed to the loss of [COOH]·, and it was therefore assumed that component 3260 could be phenylacetic acid. Co-injection of synthetic phenylacetic acid with the natural extract confirmed that component 3260 is this acid. The m/z 65, 91 and 136 ions in its mass spectrum can be explained as follows:



m/z 136 m/z 91 m/z 65

2.2.24 Anhydrides

Component 3471 (Fig. 2.86) has the very characteristic EI mass spectrum of phthalic anhydride[56]. Confirmation of this was obtained in the usual manner by co-injection of commercially available phthalic anhydride with the natural product. The presence of the prominent ions in the mass spectrum of component 3471 can be explained as follows[57]:

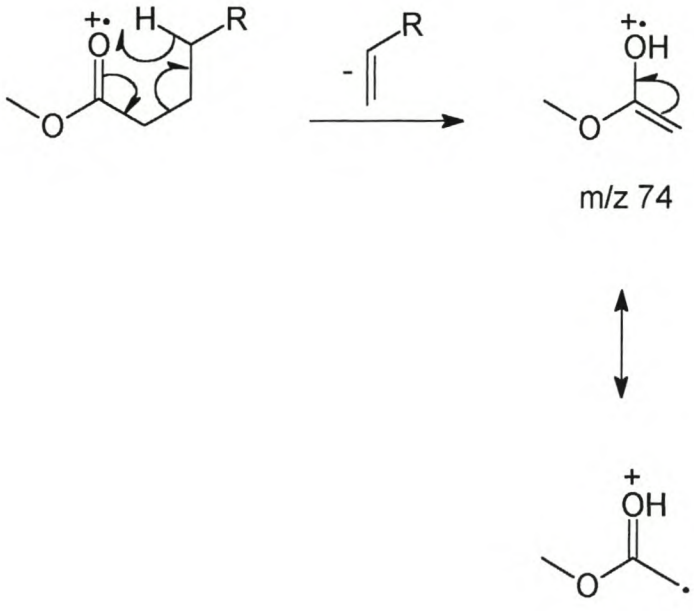


m/z 148 m/z 104 m/z 76

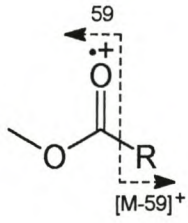
The ion at m/z 76 is most simply formulated as a benzyne ion[58,59,60].

2.2.25 Methyl esters

The EI mass spectra of components 655 (Fig. 2.87) and 1030 (Fig. 2.88) have base peaks at m/z 74. This ion is characteristic of the methyl esters of unbranched C_6 to C_{26} carboxylic acids and arises from a McLafferty rearrangement[61]:



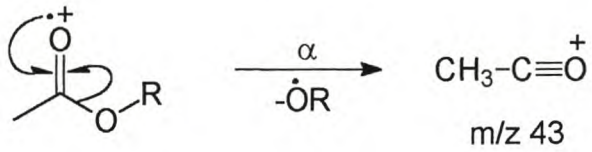
The α-cleavage ion at m/z 59 is also characteristic for these methyl esters[62]:



The inductive cleavage $[M-59]^+$ alkyl ion is normally of a low abundance except for low molecular mass methyl esters, such as methyl acetate, propionate, butanoate and pentanoate[63]. Based on the assumption that the ion at m/z 57 (35%) in the mass spectrum of component 655 could be the $[M-59]^+$ ion, this component was tentatively identified as methyl pentanoate. Co-injection of the natural secretion and a series of synthetic methyl esters (methyl butanoate through methyl heptanoate) did not only positively identify component 655 as methyl pentanoate ($C_6H_{12}O_2$, 116 Da), but also led to the identification of component 1030 as methyl hexanoate ($C_7H_{14}O_2$, 130 Da).

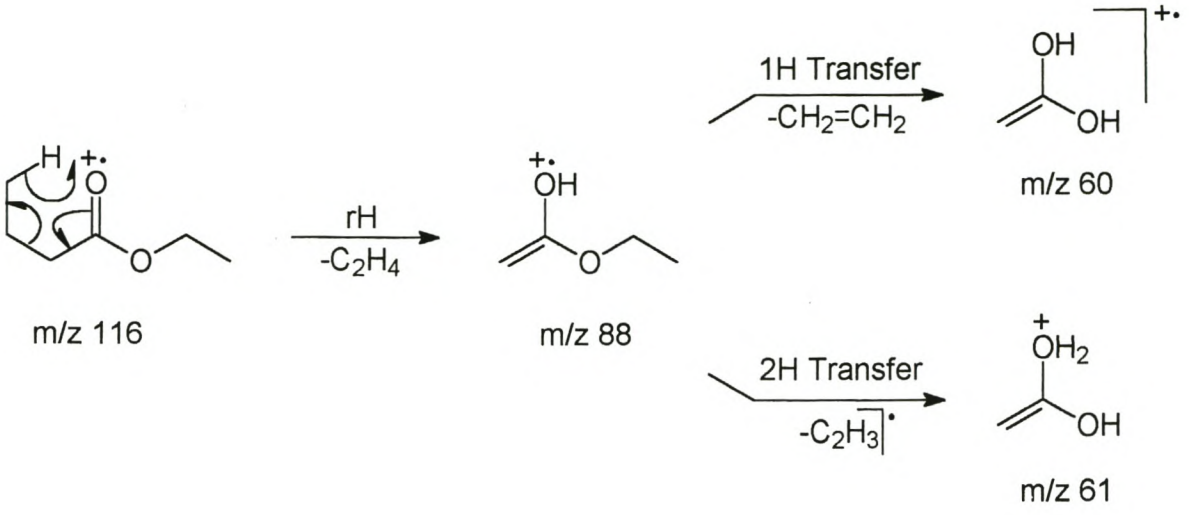
2.2.26 Ethyl and higher esters

The EI mass spectrum of component 234 (Fig. 2.89) has a significant ion at m/z 61 (15%), which is characteristic for alkyl acetates[64]. The ion is formed by rearrangement of two hydrogen atoms from the alkyl group and cleavage of the alkyl-oxygen bond[65,66]. Different processes have been suggested for the formation of this ion. According to McLafferty[67], the β and γ hydrogen atoms are principally involved in a concerted process. Black *et al.*[68] have stated that the first proton is transferred specifically from the γ position and the second one by a random selection from all the other possible positions. Benz and Biemann[69] observed that the first hydrogen atom is abstracted from the β (55%) or γ (45%) positions, and the second one from any other carbon of the alkyl chain. Djerassi and Fenselau[70] investigated butyl propionate and butyl benzoate and concluded that the two hydrogen atoms are predominantly transferred from the β and γ positions. The ion at m/z 43 can be ascribed to the formation of the acylium ion through an α-cleavage process[71]:

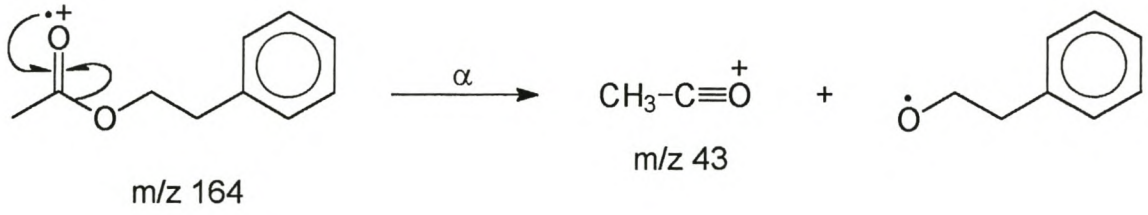


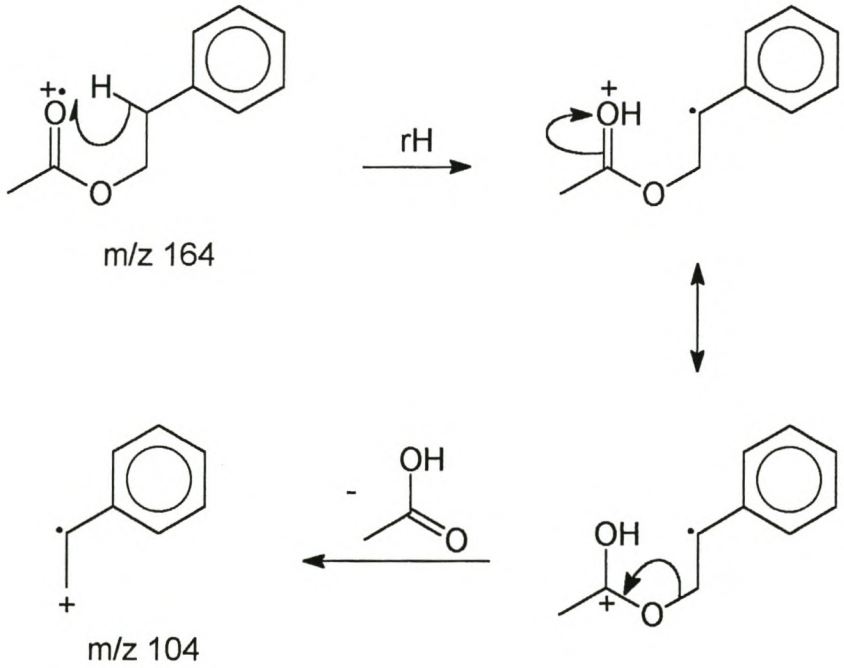
Assuming the ion at m/z 88 to be the molecular ion, component 234 has to be ethyl acetate. Co-injection of commercially available ethyl acetate with the natural extract confirmed this identification.

The EI mass spectra of components 576 (Fig. 2.90) and 924 (Fig. 2.91) each have a pair of ions at m/z 60 and m/z 61, which is characteristic for esters having acid and alcohol chains of sufficient length (ethyl butanoate or larger molecules)[72]. If the m/z 116 ion in the mass spectrum of component 576 is taken as the molecular ion, component 576 must be ethyl butanoate. This was confirmed through co-injection of the natural secretion with synthetic ethyl butanoate. The m/z 88 ion in the mass spectrum of component 924 indicates that the compound could be an ethyl ester and co-injection of a series of synthetic ethyl esters (ethyl pentanoate through ethyl octanoate) proved that component 924 is ethyl pentanoate. The ions at m/z 60 and 61, and m/z 88 in the mass spectrum of ethyl butanoate are formed as follows:

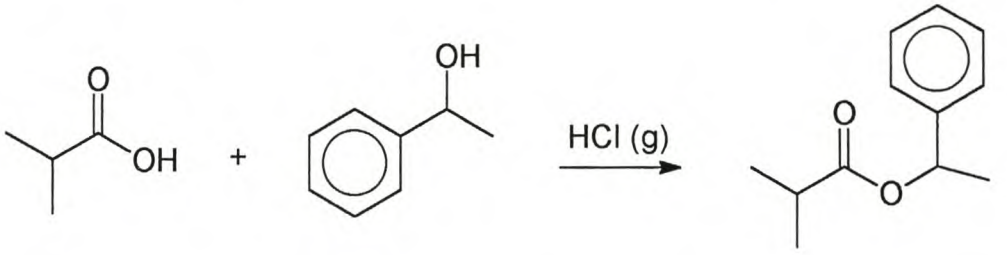


Component 2728 (Fig. 2.92) was identified as 2-phenylethyl acetate by a computer search[13] and confirmed by co-injection of synthetic 2-phenylethyl acetate with the natural extract. The prominent ions at m/z 43 and m/z 104 are formed as follows:

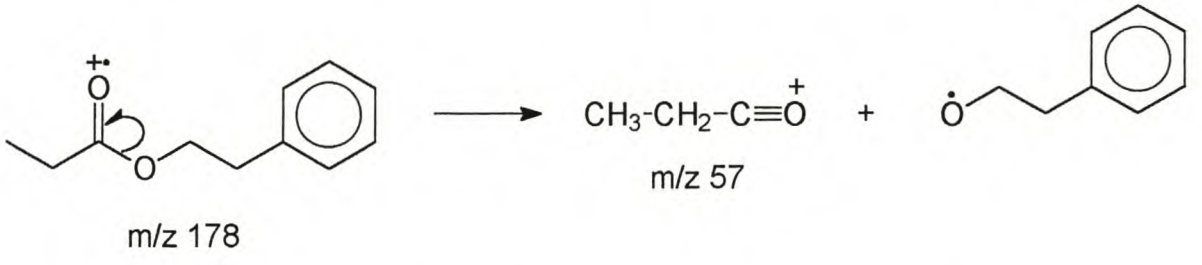




Component 2908 (Fig. 2.93) in the TIC of the interdigital secretion was identified as 1-phenylethyl 2-methylpropanoate using published mass spectra data[13]. The compound was synthesized by the simple esterification scheme shown below (see § 3.4.17 and mass spectrum Fig. 3.17) and co-injected with the natural extract, resulting in co-elution of the synthetic product with component 2908.



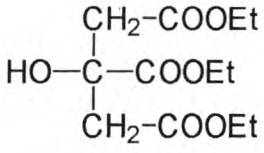
The EI mass spectra of components 3256 (Fig. 2.94) and 7202 (Fig. 2.95) are very similar and are also similar to that of 2-phenylethyl acetate (component 2728, Fig. 2.92). It was therefore assumed that these two components could also be 2-phenylethyl esters, because the mass spectra of these two components both have a base peak at m/z 104, probably formed by the same mechanism that was used to explain the presence of this ion in the spectrum of 2-phenylethyl acetate. The published mass spectra of 2-phenylethyl esters were studied[13] and it was found that 2-phenylethyl butanoate and 2-phenylethyl 2-methylpropanoate were the best candidate structures. 2-Phenylethyl propanoate was not considered because of an m/z 57 ion in its mass spectrum, the presence of which can be explained as follows:



Furthermore, the retention-time difference between 2-phenylethyl acetate (component 2728) and component 3256 is 8.8 minutes, which can be translated into a difference of more than one carbon atom. 2-Phenylethyl butanoate and 2-phenylethyl 2-methylpropanoate were synthesized [see § 3.4.18 and mass spectra Figs. 3.18(a) and 3.18(b)] and co-injection of the individual synthetic products with

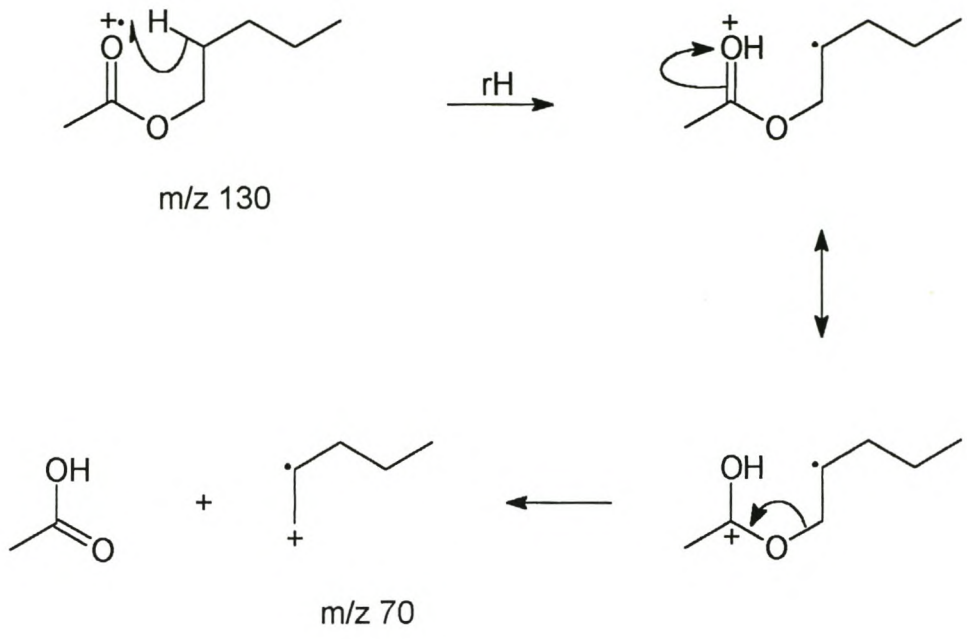
the natural extract revealed that component 2356 is 2-phenylethyl 2-methylpropanoate. The retention times of 2-phenylethyl acetate (commercially available), butanoate (synthesized), pentanoate (synthesized) and nonanoate (synthesized) were extrapolated to find a 2-phenylethyl ester with a retention time corresponding to that of component 7202. 2-Phenylethyl pentadecanoate appeared to be the most likely candidate. It was synthesized and retention-time tested. It was found that 2-phenylethyl pentadecanoate had a retention time that was too short. Including this compound in the list of model esters led to the synthesis of 2-phenylethyl hexadecanoate [see § 3.4.18 and mass spectrum Fig. 3.18(c)] as a likely candidate ester. Its mass spectrum and retention time confirmed its selection as a candidate compound and co-injection of the synthetic product with the natural extract proved that component 7202 is 2-phenylethyl hexadecanoate.

The EI mass spectrum of component 4572 (Fig. 2.96) in the interdigital secretion of the black wildebeest was identified as triethyl citrate, the structure of which is shown below, after a computer search[13] indicated this compound as a likely possibility. This was confirmed in the usual manner by co-injecting commercially available triethyl citrate with the natural product.

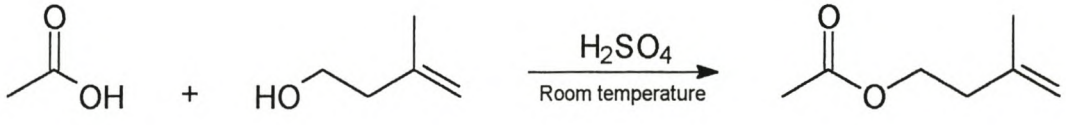


2.2.27 Unsaturated esters

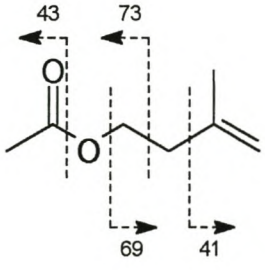
The EI mass spectrum of component 902 (Fig. 2.97) displays three ions of interest, namely m/z 43, 61 and 68. It was assumed that the m/z 43 and m/z 61 ions are indicative of an alkyl acetate, as was discussed in § 2.2.26 for ethyl and higher esters. A mixture of synthetic acetates (ethyl through *n*-hexyl acetate) was injected and it was found that component 902 had a retention time between that of *n*-butyl and *n*-pentyl acetate. This meant that component 902 could be a C_5-acetate with either branching, unsaturation, or both, in the C_5-alcohol moiety of the ester. The m/z 68 ion suggests the presence of a double bond, because the mass spectrum of *n*-pentyl acetate contains an m/z 70 rearrangement ion:



As discussed previously, acetic acid (component 367) and 3-methyl-3-buten-1-ol (component 525) were found in the extract of the interdigital secretion of the black wildebeest. The ester of these two compounds, 3-methyl-3-butenyl acetate, was therefore synthesized by the acid-catalyzed esterification shown below (see § 3.4.19 and mass spectrum Fig. 3.19). Co-injection of the synthetic product with the natural secretion proved that component 902 is this ester.

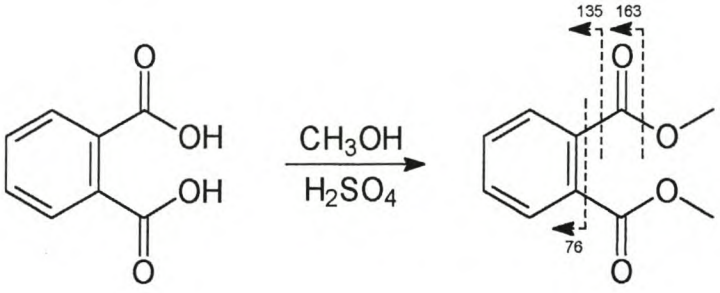


Some of the other ions present in the mass spectrum of component 902 are formed by the following simple α-cleavages:

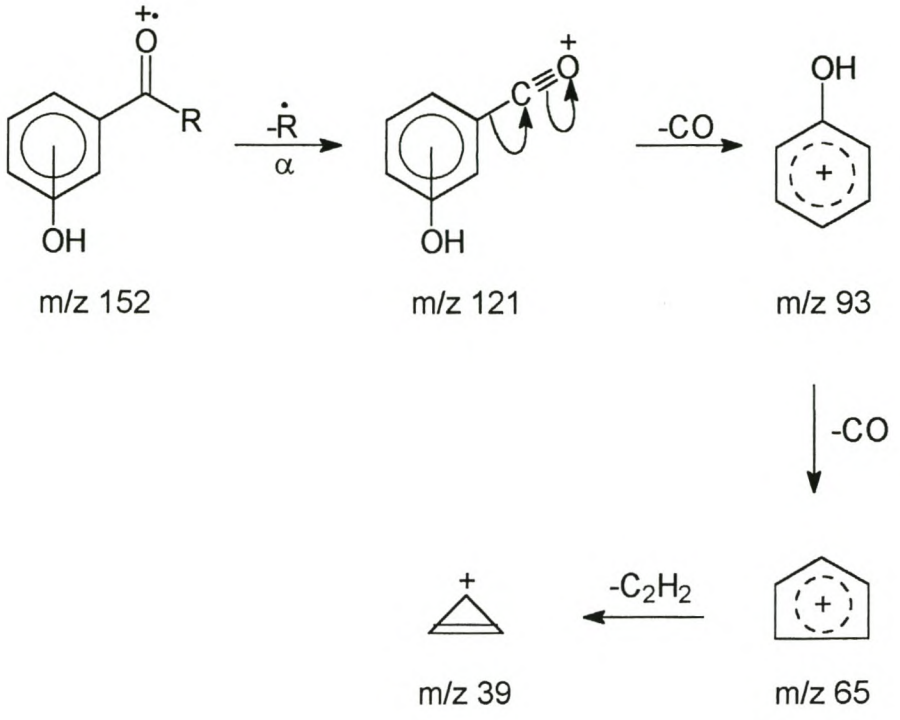


2.2.28 Aromatic esters

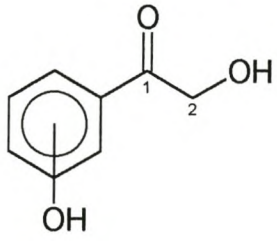
Component 3797 (Fig. 2.98) was identified as dimethyl phthalate in the following way: The m/z 194 ion in the EI mass spectrum of this component was assumed to be the molecular ion and the m/z 163 ion (base peak) was assumed to be formed by the loss of a methoxyl radical $[OCH_3]^{\cdot}$. There is also an $[M\text{-}59]^+$ ion at m/z 135 that can be ascribed to the loss of a carbomethoxyl radical $[COOCH_3]^{\cdot}$ from the molecular ion and an $[M\text{-}118]^+$ ion at m/z 76 that can be ascribed to the loss of two carbomethoxyl radicals from the molecular ion. This is consistent with what is reported for dimethyl phthalate in Budzikiewicz, Djerassi and Williams[73] and was confirmed by co-injection of the natural extract and synthetic dimethyl phthalate prepared from phthalic acid and excess methanol as shown below (see § 3.4.20 and mass spectrum Fig. 3.20).



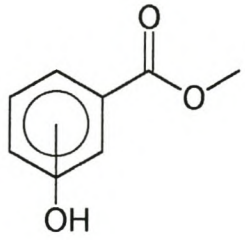
The EI mass spectrum of component 4385 (Fig. 2.99) has the same general appearance as the mass spectra of 4-hydroxybenzaldehyde (component 4220, Fig. 2.41) and 4-hydroxyacetophenone (component 4456, Fig. 2.56), already identified, *viz.* a series of ions at m/z 39, 65 and 93 and a base peak at m/z 121. If it is assumed that these ions in component 4385 are of the same composition as those in 4-hydroxybenzaldehyde and 4-hydroxyacetophenone, the following general mechanism can be formulated:



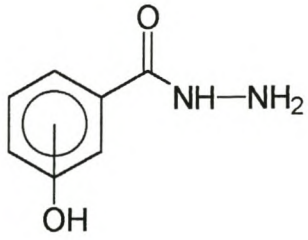
The loss of 31 atomic mass units (R) from the molecular ion at m/z 152 to give the m/z 121 ion could be attributed to the loss of one of the following fragments: $[CH_2OH]^{\cdot}$, $[OCH_3]^{\cdot}$ or $[NHNH_2]^{\cdot}$. The parent compound could therefore be one of the following compounds:



1-(Hydroxyphenyl)-
2-hydroxyethanone

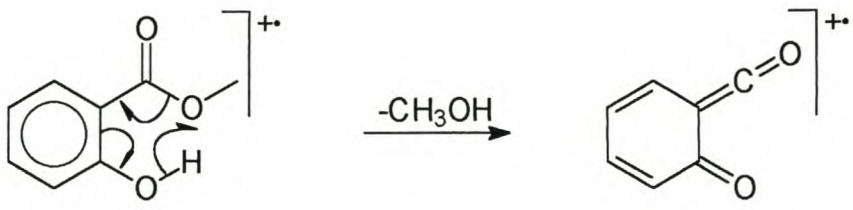


Methyl
hydroxybenzoate



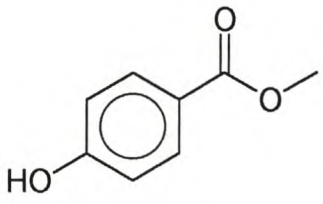
Hydroxybenzoic
hydrazide

The methyl hydroxybenzoates were considered first, but methyl 2-hydroxybenzoate was not retention-time tested because it has an m/z 120 base peak[74]:



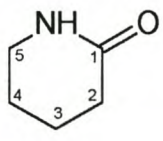
m/z 152 m/z 120

Retention-time testing of the other two commercially available isomers indicated that methyl 4-hydroxybenzoate was the most likely candidate structure, and co-injection of this compound with the natural interdigital extract proved that component 4385 is methyl 4-hydroxybenzoate:

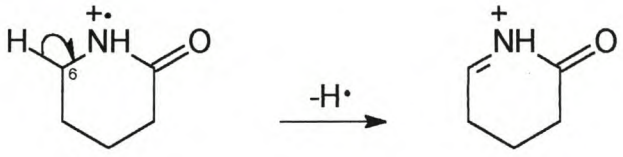


2.2.29 Lactams

The EI mass spectrum of component 2982 (Fig. 2.100) has an ion of uneven mass at m/z 99. If the retention time of the compound is taken into consideration, it seems reasonable to assume that this ion could be the molecular ion of the compound, in which case the compound must contain an uneven number of nitrogen atoms[75]. According to a computerized library search[13], this compound is 2-piperidone (bp 256°C). As this compound contains one nitrogen atom and has a molecular mass of 99 Da, the result of the Wiley library search[13] was accepted as a working premise. 2-Piperidone, also known as 5-aminopentanoic acid lactam, δ-valerolactam or 5-pentanelactam, has the following structure:

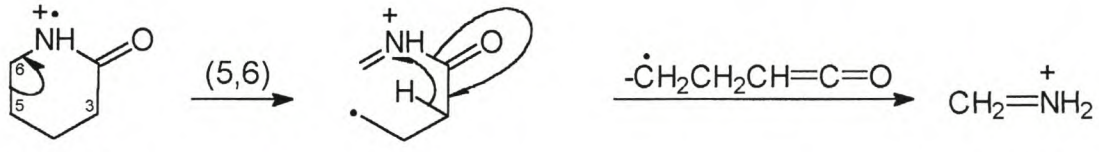


The strong molecular ion is accompanied by a somewhat less abundant $[M-1]^+$ ion (20%). This is probably due to elimination of a C6 hydrogen atom to produce an immonium ion[76]:



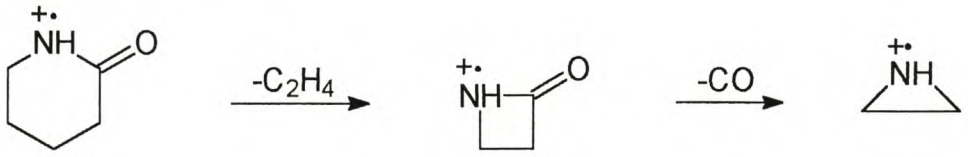
m/z 99 m/z 98

The ion at m/z 30 can be rationalized by initial α-cleavage of the C5-C6 bond, followed by hydrogen transfer from C3 and nitrogen-carbon bond fission in the following manner[76]:



m/z 99 m/z 30

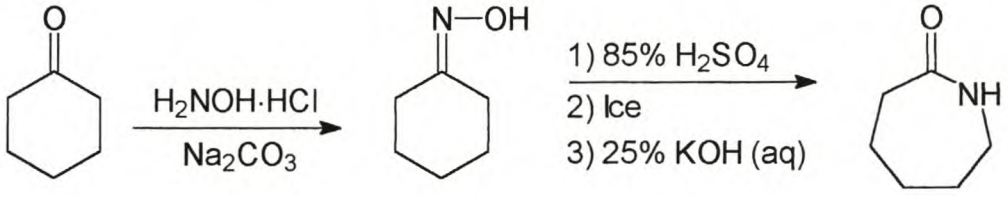
The ions at m/z 55 $[C_4H_7]^+$ and m/z 56 $[C_4H_8]^{+\cdot}$ can be attributed to the loss of $[NH_2CO]^{\cdot}$ (44 atomic mass units) and HNCO (43 atomic mass units) from the molecular ion, respectively, while those at m/z 41 and m/z 42 represent the fragments formed following a further loss of CH_2 from the aforementioned ions. As several mechanisms can be proposed for the formation of the $[M-29]^+$ ion at m/z 70, the presence of this ion in the spectrum cannot be explained in the absence of corroborating evidence. Two further ions at m/z 71 and m/z 43 can be rationalized in terms of the ejection of ethylene from the molecular ion, followed by a further loss of CO, in the following manner[77]:



m/z 99 m/z 71 m/z 43

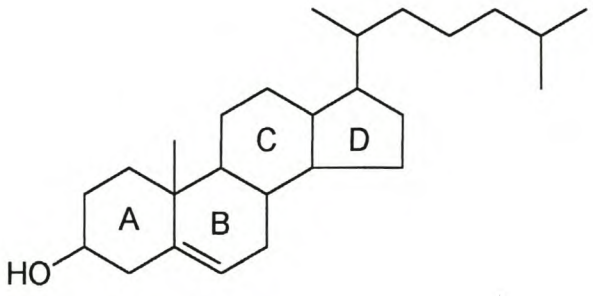
Co-injection of the commercially available compound with the natural extract confirmed the identification of component 2982 as 2-piperidone.

The retention time of component 3227 (Fig. 2.101) is about 4 minutes longer than that of 2-piperidone, component 2982. This, together with the fact that component 3227 also appears to have a molecular ion of uneven mass at m/z 113, suggesting the presence of an uneven number of nitrogen atoms, as well as the presence of ions at m/z 30, 43, 55 and 56 ions and the even mass ion at m/z 84 $[M-29]^+$, led to the conclusion that this component could be a homologue of the already discussed 2-piperidone. The next homologue in the series of saturated lactams is 6-hexanelactam (6-caprolactam, ε-caprolactam or hexane-6-lactam), with a molecular mass of 113 Da. 6-Hexanelactam was synthesized according to the scheme shown below [see § 3.4.21 and mass spectrum Fig. 3.21(b)]. The mass spectrum of the synthetic and natural compound were identical. The identification was confirmed by co-injection of the synthetic product and the natural extract.



2.2.30 Steroids

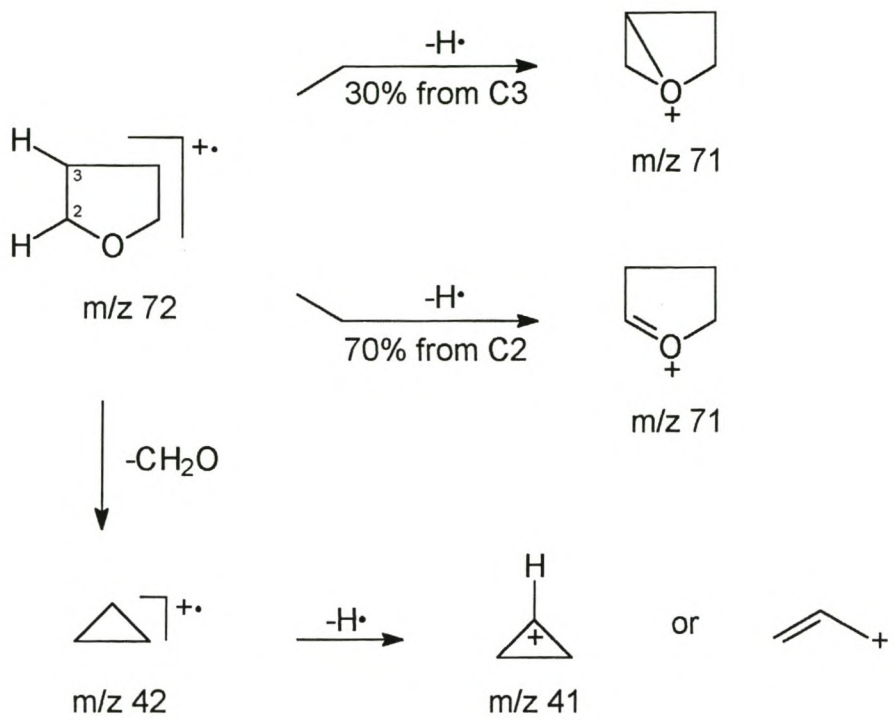
A major component of the interdigital secretion of the black wildebeest, component 8805, has an EI mass spectrum (Fig. 2.102) with a steroid like appearance, a molecular ion at m/z 386, fragment ions at m/z 371 $[M-CH_3]^+$, 368 $[M-H_2O]^{+\cdot}$ and 353 $[M-CH_3-H_2O]^+$, and prominent ions at m/z 247 and m/z 301. These ions are all characteristic for cholesterol and co-injection of commercially available cholesterol with the natural extract confirmed that component 8805 is cholesterol, the structure of which is shown below.



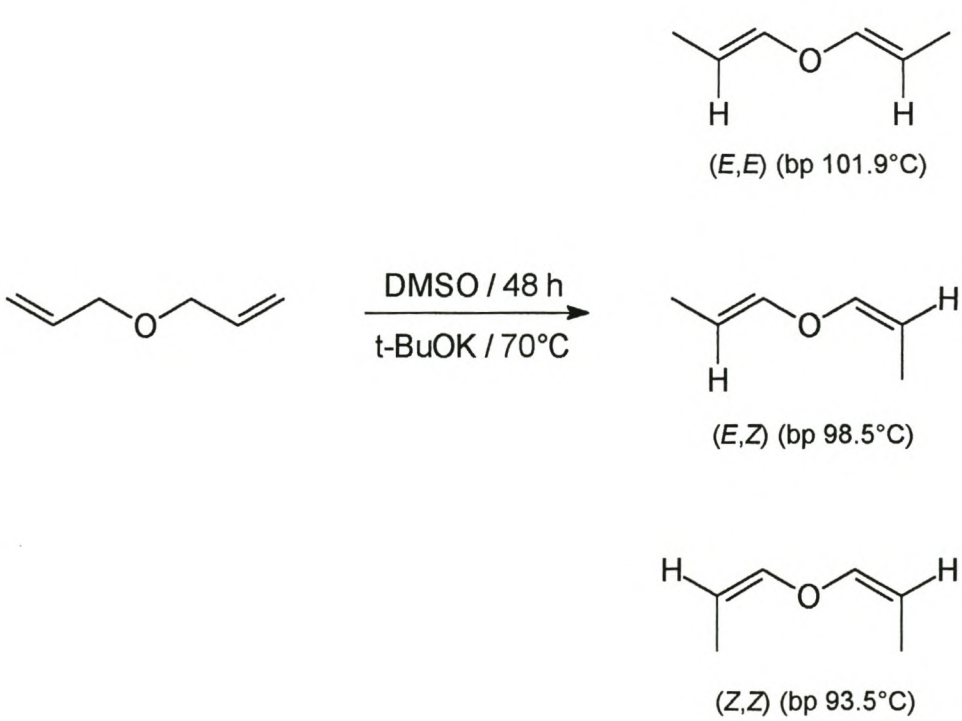
The mass spectra of steroids with saturated ring systems have $[M-72]^{+\cdot}$ ions due to the elimination of ring A from the molecular ion[78]. Cholesterol, however, does not have this m/z 314 ion because the double bond in ring B stabilizes ring A. The presence of the double bond also leads to the formation of an ion at m/z 255, representing the ionized steroid nucleus formed by the loss of the alkyl side chain and water. The ion at m/z 213 is formed through loss of the alkyl side chain, water and ring D from the molecular ion. Loss of the alkyl side chain and ring D from the molecular ion produces m/z 231[79].

2.2.31 Miscellaneous

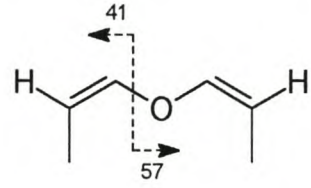
Component 232 (Fig. 2.103) was tentatively identified as tetrahydrofuran by comparison of its mass spectrum with published spectra[80]. The structure of component 232 was confirmed through co-injection of synthetic tetrahydrofuran with the natural extract. The formation of the ions at m/z 41, 42 (base peak), 71 and 72 (molecular ion) in the spectrum can be explained as follows:



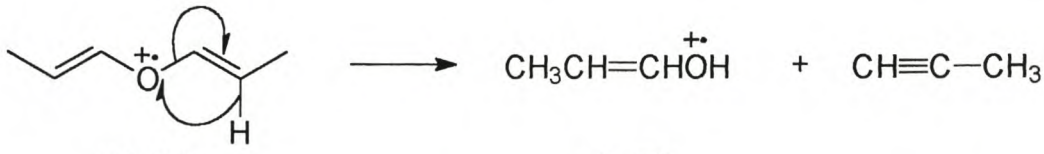
Component 307 (Fig. 2.104) was tentatively identified as dipropenyl ether using the Wiley library[13]. Since this compound has three geometric isomers, namely (*Z*,*Z*)-, (*E*,*Z*)- and (*E*,*E*)-dipropenyl ether, it was necessary to synthesize these unsaturated ethers [see § 3.4.22 and mass spectra Figs. 3.22(a), 3.22(b) and 3.22(c)] using allyl ether as reagent according to the following scheme:



The GC-MS analysis of the reaction mixture showed all three isomers and since they elute according to boiling points in the order (*Z*,*Z*), (*E*,*Z*) and (*E*,*E*), it was possible by co-injection of the synthetic product with the natural extract to prove that component 307 is the (*Z*,*Z*)-isomer. The formation of the ions at m/z 41 and m/z 57 can be explained as follows:

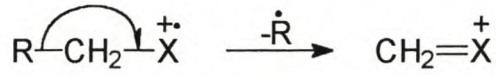


The m/z 58 ion in the EI mass spectrum of this component is formed by migration of a β-hydrogen atom to the oxygen atom as follows[81,82]:

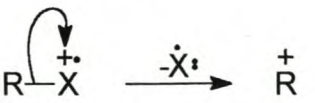


m/z 98 m/z 58

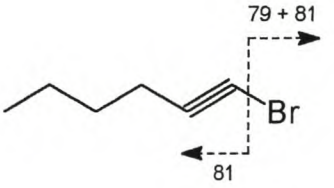
Component 853 displays a characteristic isotope pattern for bromine (^{79}Br : ^{81}Br = 100 : 97.3) in its EI mass spectrum (Fig. 2.105) at m/z 160 (~11%) and m/z 162 (~10.5%). The same pattern is repeated at m/z 145 (5%) and m/z 147 (5%), which could be due to the loss of a methyl radical $[M\text{-}CH_3]^+$. The large electron affinity of halogen atoms (F > Cl > Br > I) is the reason why α-cleavage, with charge retention by the heteroatom fragment, usually only results in the formation of ions with low abundance:



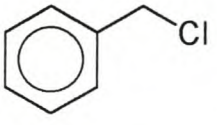
The two-electron shift (heterolytic cleavage), with loss of a halogen atom is generally preferred to α-cleavge[83]:



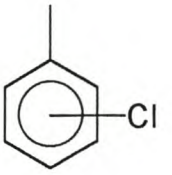
If it is assumed that the base peak at m/z 81 is due to the loss of the bromine radical (160 – 79 = 162 – 81 = 81 Da), the m/z 81 ion must be $[C_6H_9]^+$, which indicates that the component under discussion could be a bromohexyne, bromohexadiene or bromocycloalkene. To get an idea of the retention times of these compounds, commercially available 1-bromohexane, 1-bromohexyne and bromobenzene were injected and it was found that 1-bromohexyne had the correct retention time. Co-injection of 1-bromohexyne with the natural interdigital extract proved that component 853 is 1-bromohexyne. The formation of the ions at m/z 79 and m/z 81 can be explained as follows:



Component 1541 (Fig. 2.106(a)) displays the familiar tropylium ion at m/z 91 (base peak), a molecular ion at m/z 126 and an $[M+2]^{+\cdot}$ ion at m/z 128 in a ratio typical for a compound containing one chlorine atom. The loss of 35 and 37 atomic mass units from the molecular ions at m/z 126 and m/z 128, to give the m/z 91 ion, could only be due to the loss of the two chlorine isotopes, namely ^{35}Cl (100%) and ^{37}Cl (32%), respectively, so that component 1541 must be either benzyl chloride or one of the chlorotoluenes:

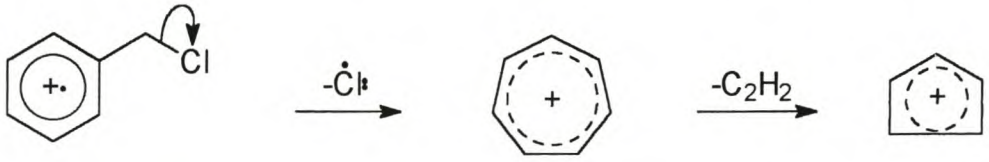


Benzyl chloride
bp 177-181°C



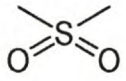
Chlorotoluene
bp 157-162°C

Synthetic benzyl chloride was co-injected with the natural extract and co-eluted with component 1541, proving that component 1541 is benzyl chloride. The m/z 83 (98%) and m/z 111 (15%) ions in the EI mass spectrum of this component in the male secretion are probable due to another component eluting together with component 1541. The m/z 83 (13%) ion has a much lower relative abundance and there is no ion present at m/z 111 in the female's mass spectrum (Fig. 2.106(b)), component 1528. The m/z 91 and m/z 65 ions can be explained as follows:



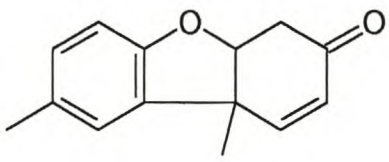
m/z 126 + 128 m/z 91 m/z 65

The EI mass spectrum of component 1922 (Fig. 2.107) displays a molecular ion at m/z 94 (55%) and a base peak at m/z 79 $[M\text{-}CH_3]^+$. This correlates with the published mass spectrum[84] for dimethyl sulfone (bp 238°C), the structure of which is shown below.

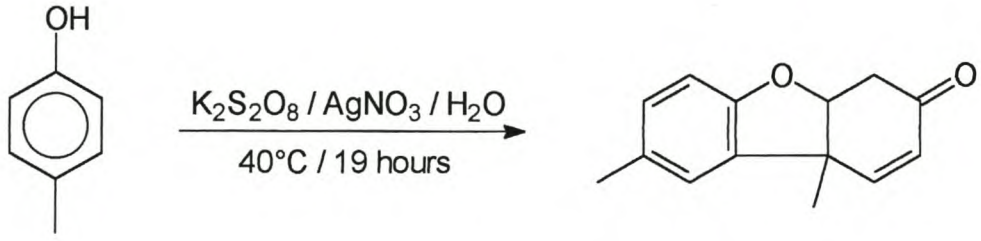


This sulfur-containing compound has been found in certain moss[85] and plant[86] species, in cow blood[87], the adrenal kidney[88] and in the dorsal secretion of the springbok, *Antidorcas marsupialis*[89], so that it was thought that it could also possibly be present in the interdigital secretion of the black wildebeest. Co-injection of commercially available dimethyl sulfone with the natural extract confirmed this.

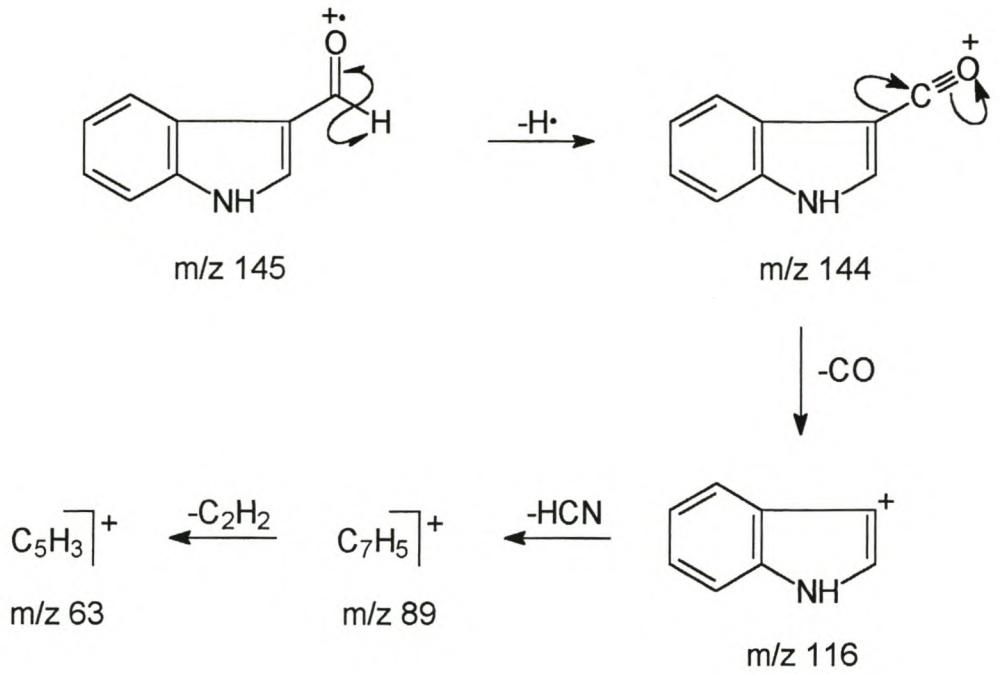
Component 5177 (Fig. 2.108) was identified as Pummerer's ketone on the basis of a computerized library search[13] which listed the ketone as a possibility:



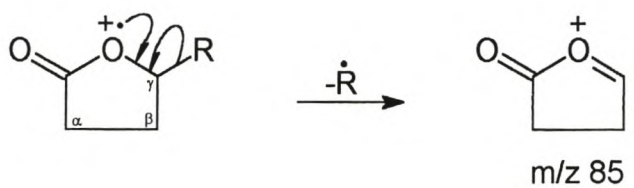
The ketone was synthesized (see § 3.4.23 and mass spectrum Fig. 3.23) by the silver nitrate catalyzed persulphate oxidation of *p*-cresol as shown below. Co-injecting the secretion and one of the oxidation products, identified as Pummerer's ketone by GC-MS analysis, confirmed the identification.



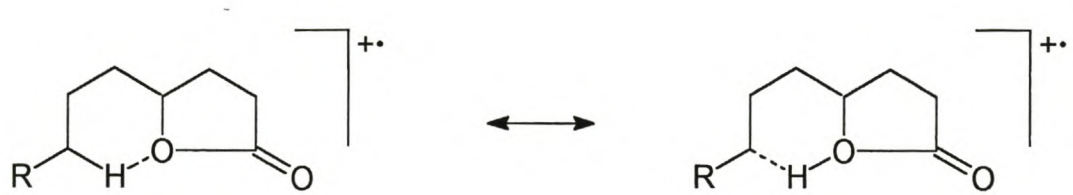
The EI mass spectrum of component 5877 (Fig. 2.109) has a base peak at m/z 144 and a molecular ion at m/z 145. The very abundant $[M-1]^+$ ion at m/z 144 indicates an aromatic type aldehyde and the uneven molecular mass indicates an uneven number of nitrogen atoms in the molecular formula, which could be C_9H_7NO, $C_7H_{15}NO_2$ or $C_6H_{11}NO_3$. The loss of 29 atomic mass units from the molecular ion to give the m/z 116 ion could be due to the loss of CHO. The difference between the ions at m/z 116 and m/z 89 is 27 atomic mass units, equivalent to the loss of HCN and the ion at m/z 89 could then be ascribed to $[C_7H_5]^+$, which could be taken as evidence in favour of this compound being an oxygen- or nitrogen-containing heterocycle[44]. This would then indicate that this compound contains one oxygen and one nitrogen atom, with C_9H_7NO as its molecular formula. Library searches[13] gave 1*H*-indole-3-carboxaldehyde (3-indolealdehyde) as a very likely candidate and co-injection of the natural extract with the synthetic compound proved that component 5877 is indeed 1*H*-indole-3-carboxaldehyde. Some of the ions under discussion are formed as follows:



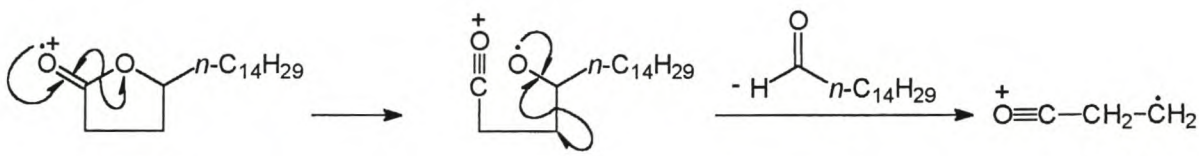
The EI mass spectrum of component 6593 (Fig. 2.110) has its base peak at m/z 85. This ion is characteristic of, amongst others, γ-substituted γ-lactones, and its formation can be explained in terms of the loss of the γ-substituent[90,43]:



The molecular ion was not detected, as can be expected, since, in general, only a small fraction of the total ion current is carried by the molecular ion[91] in the case of γ-lactones. γ-Lactones with side chains of three or more carbon atoms can expel water from the molecular ion, since the spatial conformation of the ion allows hydrogen transfer *via* the following cyclic six-membered transition state[43]:



This hydrogen transfer destabilizes the lactone ring, which leads to an additional hydrogen transfer and the elimination of a molecule of water. The elimination of a second water molecule is also possible. If the ions at m/z 264 and m/z 246 are taken as $[M\text{-}H_2O]^{+\cdot}$ and $[M\text{-}2H_2O]^{+\cdot}$, respectively, the molecular mass of component 6593 must be 282 Da. If it is further assumed that the side-chain is unbranched, component 6593 could be 4-octadecanolide. Co-injection of the commercially available compound with the natural interdigital extract confirmed its identification as 4-octadecanolide. The ion at m/z 56 in its mass spectrum can be explained as follows[92]:

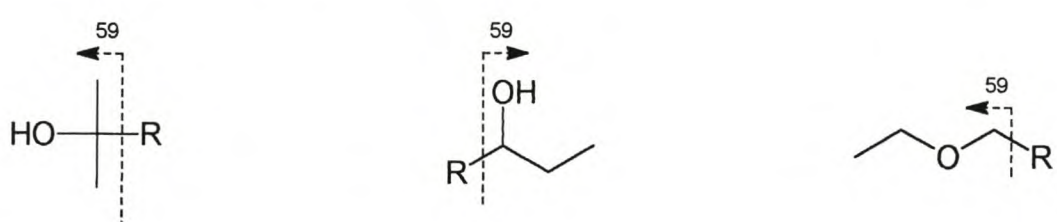


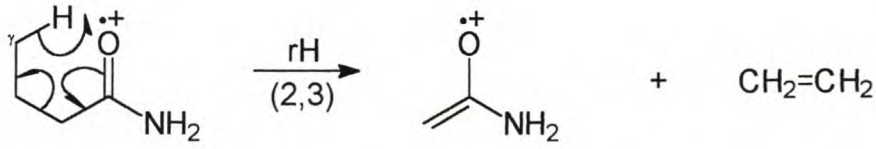
m/z 282 m/z 56

2.2.32 Unidentified components

Some of the constituents of the interdigital secretion of the black wildebeest remained unidentified, mainly because the available mass spectral data do not allow their unequivocal identification. The conclusions drawn from retention-time comparisons are nevertheless discussed. The major problem was that many of the reference compounds were not readily available for retention-time testing and in most cases the synthesis of a large number of reference compounds was considered to lie outside the scope of the present investigation.

The EI mass spectrum of component 435 (Fig. 2.111) has a base peak at m/z 59, which is indicative of 2-methylalkan-2-ols, alkan-3-ols, ethyl ethers and acid amides with a γ-hydrogen atom, as is shown below:

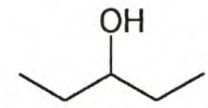




m/z 59

Retention-time comparison with primary alcohols showed that component 435 has a retention time between 1-butanol and 1-pentanol and it was therefore assumed that this component could be a branched pentanol. The possibilities were therefore:

2-Methyl-2-butanol

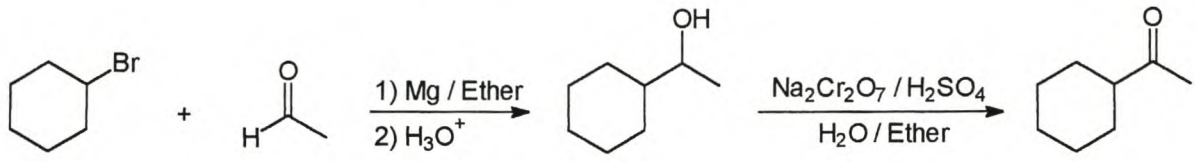


3-Pentanol

Only commercial available 3-pentanol (bp 116°C) was retention-time tested, because 2-methyl-2-butanol has ions at m/z 73 $[M\text{-}CH_3]^+$ (40-70%)[13] and m/z 55 $[M\text{-}CH_3\text{-}H_2O]^+$ (30-60%)[13]. It was found that 3-pentanol has a retention time only seconds shorter than component 435. When investigating the ethers, the boiling point of 3-pentanol was used as a guideline, since 3-pentanol has a retention time just seconds shorter than that of component 435 and because the column in use separates compounds to a great extent according to their boiling points. This showed that C_5- and C_6-ethers would have too short retention times and C_8-ethers too long retention times. The C_7-ethers were investigated and although ethyl pentyl ether (bp 119-120°C) and ethyl 3-methylbutyl ether (bp 112-113°C) have boiling points in the correct range and base peaks at m/z 59, they also have ions at m/z 55 and m/z 70, that are not present in component 435. The other C_7-ethyl ethers were not considered because they would all have boiling points lower than that of ethyl 3-methylbutyl ether. The acid amides were not considered, because they have ions at m/z 44 $[CONH_2]^{+\cdot}$ and because the first amide which has a γ-hydrogen atom is butanamide with a boiling point of 216°C. This component remained unidentified.

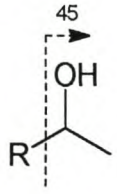
Computer searches[13] indicated that component 603 (Fig. 2.112) could either be an unsaturated ketone or a cycloalkyl methyl ketone. If the ion at m/z 126 is assumed to be the molecular ion, the compound could have the molecular formula $C_8H_{14}O$. Commercially available 6-methylhept-5-en-2-one was retention-time tested

and found to have a retention time that was approximately 2.7 times too long. The unsaturated C_7-ketone, 5-methylhex-5-en-2-one, was also tested and since this compound was found to have a retention time 1.7 times too long, it was thought that all the octenones would have too long retention times to be component 603. Acetylcyclohexane (1-cyclohexylethanone) was synthesized according to the scheme shown below [see § 3.4.24 and mass spectrum Fig. 3.24(b)] and retention-time tested, but was found to have a retention time 3.1 times too long.

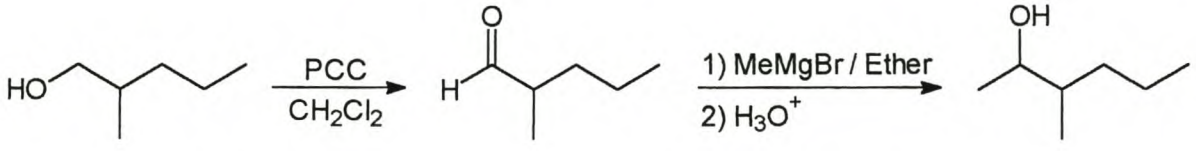


This component also remained unidentified.

The EI mass spectrum of component 757 (Fig. 2.113) has a base peak at m/z 45 and was therefore at first assumed to be an alkan-2-ol:

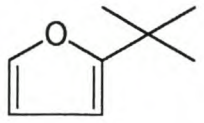


Component 757 has a retention time between that of 2-hexanol and 2-heptanol and could therefore be a branched 2-heptanol. 3-Methyl-2-hexanol was synthesized as follows [see § 3.4.25 and mass spectrum Fig. 3.25(b)] and found to have a too long retention time.



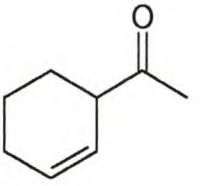
This meant that 4- and 5-methyl-2-hexanol would also have retention times too long. No further work was done on this component.

The EI mass spectrum of component 793 (Fig. 2.114) has the same general appearance as that of *trans*-6-methyl-3,5-heptadiene-2-one (component 2131, Fig. 2.51), the only difference being that the relative abundance of the ions differ. On the basis of the general discussion of the identification of component 2131, the following compounds were retention time tested.



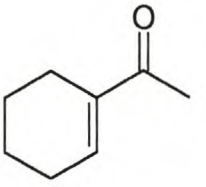
2-*tert*-Butylfuran

Retention time 0.5 times too short



3-Acetyl-1-cyclohexene

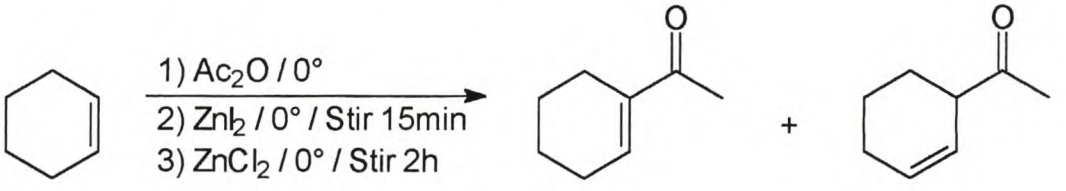
Retention time 2.4 times too long



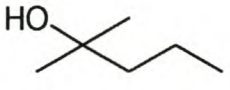
1-Acetyl-1-cyclohexene

Retention time 3.0 times too long

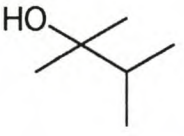
1-Acetyl- and 3-acetyl-1-cyclohexene were synthesized as follows [see § 3.4.26 and mass spectra Figs. 3.26(a) and 3.26(b)]:



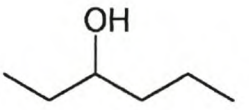
Component 888 (Fig. 2.115) has a base peak at m/z 59, which was, as a first assumption, taken to be formed from a 2-methylalkan-2-ol or an alkan-3-ol. After retention-time comparison of component 888 and a series of primary alcohols, it was found that it had a retention time between that of 1-pentanol (bp 137°C) and 1-hexanol (bp 156.5) and could therefore be one of the following branched hexanols:



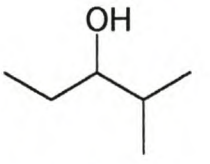
bp 120-122°C



bp 120-121°C



bp 135°C

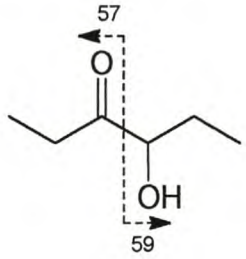


bp 128°C

Both 3-hexanol and 2-methyl-2-pentanol have retention times too short to be component 888, and therefore 2-methyl-3-pentanol and 2,3-dimethyl-2-butanol were not considered because they are branched and have lower boiling points, and therefore even shorter retention times. Next, the heptanols were considered, with the following results: 2-methyl-2-hexanol had a too short retention time, which meant that 2,3- and 2,4-dimethyl-2-pentanol, and 2,3,3-trimethyl-2-butanol would also have retention times that are too short. 3-Heptanol and 4-methyl-3-hexanol had retention times that are too long, and the retention times of 5-methyl-3-hexanol and 2,2-dimethyl-3-pentanol were too short. This component remained unidentified.

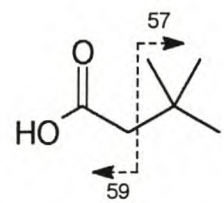
The m/z 43, 57 and 58 ions, together with the m/z 114 ion, assumed to be the molecular ion, in the EI mass spectrum of component 998 (Fig. 2.116), led to the conclusion that this component could be a 2-heptanone. Commercially available 2-heptanone (bp 149-150°C) was injected and found to have a retention time 10 seconds too short. This also meant that component 998 could not be a branched 2-heptanone. Commercially available heptanal (bp 153°C), although not having a mass spectrum similar to that of component 998, was injected because it has a boiling point slightly higher than that of 2-heptanone and was found to have a retention time within a second of component 998. The major problem with this component was that it is a shoulder on the peak of *meso*-2,3-butanediol, component 988, a component with a peak area 25 times larger than that of component 998, and therefore it was difficult to obtain a pure mass spectrum. The assumption that m/z 114 is the molecular ion, was also in doubt. No further work was done on this component.

Component 1138 (Fig. 2.117) was thought to be 4-hydroxy-3-hexanone (propionoin), shown below, on the basis of a computer search[13], and because four other aliphatic hydroxy ketones had already been identified in the natural secretion (see § 2.2.18).



4-Hydroxy-3-hexanone was synthesized (see § 3.4.27 and mass spectrum Fig. 3.27(d)), injected and found to have a retention time almost identical to that of component 1138. A correlation between the mass spectra of component 1138 and synthetic 4-hydroxy-3-hexanone, however, revealed significant differences, for example, differences in the abundance of the m/z 31 and m/z 43 ions and the absence of a molecular ion at m/z 116 in the mass spectrum of component 1138. The ion at m/z 143 in the mass spectrum of component 1138, which was initially ignored, could also not be explained. A single ion chromatogram showed that this ion belonged to component 1138 and there was no evidence that the m/z 143 ion belonged to a compound eluting together with component 1138. It was therefore concluded that component 1138 could not be 4-hydroxy-3-hexanone. The branched compound, 2-hydroxy-2-methyl-3-pentanone, was not considered because this compound would have a retention time shorter than that of 4-hydroxy-3-hexanone, which was already shown to have a retention time very close to that of component 1138. No further work was done on this component.

3,3-Dimethylbutanoic acid (bp 185-190°C) was the only compound tested in an attempt to identify component 1240 (Fig. 2.118):

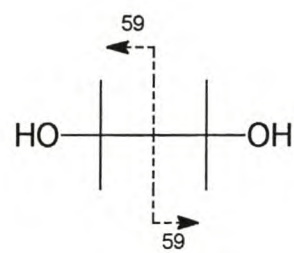


This acid, however, had a retention time 1.4 minutes longer than component 1240. On closer inspection, the ions at m/z 149 and m/z 151 were found to have an abundance ratio of 2.93 : 1, which is the same as that of the two chlorine isotopes (^{35}Cl : ^{37}Cl = 100 : 34.1 ≈ 2.93 : 1). It could, however, not be said with certainty that these two ions belong to component 1240 or whether they belong to another component eluting together with component 1240. No further work was done on this component.

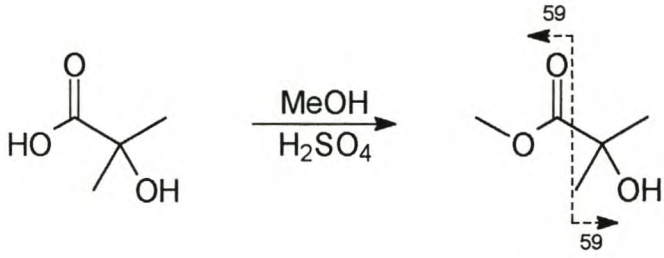
The EI mass spectrum of component 1315 (Fig. 2.119) has the typical ions of a ketone, namely m/z 43 (base peak), 57, 58, 71, 72, 85 and 86, the even ions probably being McLafferty rearrangement ions. If it is assumed that the m/z 128 ion is the molecular ion, component 1315 could be an octanone. Components 1436 and

1269 have previously been identified as 2-octanone and 6-methyl-2-heptanone, respectively, and after studying the published mass spectra[13] of 3-, 4- and 5-methyl-2-heptanone, it was concluded that component 1315 could not be one of these compounds. It was also assumed that all the other branched 2-octanones would have retention times too short to be component 1315. Component 1169 had been identified as 3-octanone, and since this ketone has a retention time shorter than that of component 1315, component 1315 could not be one of the branched 3-octanones. Synthetic 4-octanone has a mass spectrum very similar to that of component 1315, but this ketone's retention time is 0.3 minutes too short, which also means that all the branched 4-octanones would have retention times that are too short. No further work was done on this component.

As a starting point, component 1386 (Fig. 2.120) was assumed to be an alcohol, because of its base peak at m/z 59. The retention time of component 1386 is approximately the same as that of 1-heptanol (bp 176°C), and therefore this component was assumed to be a heptanol or octanol. Commercially available 3-heptanol, 2-methyl-2-heptanol and 3-octanol were injected and it was found that the first two alcohols have retention times shorter and the third alcohol a retention time longer than component 1386. This meant that all the branched 3-heptanols and all the branched 2-methyl-2-hexanols and 2-methyl-2-heptanols would have too short retention times, leaving the branched 3-octanols as possible candidate structures. The methyl-3-heptanols all have m/z 101 $[M-29]^+$ ions due to α-cleavage in their mass spectra and since this ion is absent in the mass spectrum of component 1386, these methyl-3-heptanols were not considered. Pinacol (2,3-dimethyl-2,3-butanediol), shown below, was found to have a retention time 2.3 minutes too short:

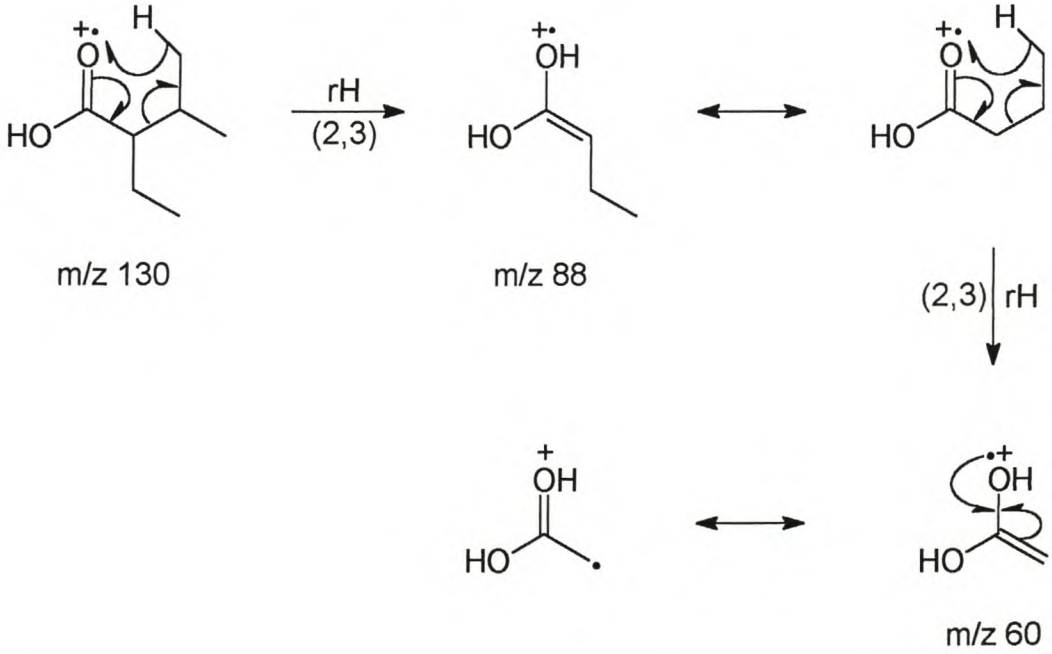


A final compound that was considered as a candidate for component 1386 is methyl 2-hydroxy-2-methylpropanoate (see § 3.4.28 and mass spectrum Fig. 3.28), synthesized according to the scheme shown below:



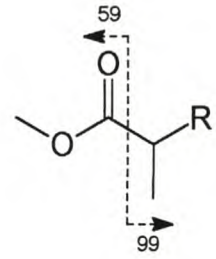
This methyl ester has a retention time that is too short for it to be component 1386. No further work was done on this component.

The EI mass spectrum of component 1718 (Fig. 2.121) has a general appearance similar to that of 2-ethylbutanoic acid (component 1559, Fig. 2.81), with ions at m/z 43, 55, 73 and 88. Injection of synthetic 2-ethylpentanoic acid showed that component 1718 had a retention time between these two acids, which leaves only one possible option, namely 2-ethyl-3-methylbutanoic acid. This branched acid has two γ-hydrogen atoms, and therefore two consecutive McLafferty rearrangements are possible, giving an m/z 60 ion:



This m/z 60 ion which is a product of a double McLafferty rearrangement, is not present in the mass spectrum of component 1718 and consequently this acid was not further considered. Although there is an m/z 88 McLafferty rearrangement ion in the mass spectrum under discussion, the absence of ions at m/z 60 and m/z 61 excludes

this component from being an ethyl ester, as was explained in § 2.2.26. Another compound type that gives m/z 88 ions is the methyl 2-methylalkanoates. Injection of a series of synthetic methyl esters showed that component 1718 lies between methyl heptanoate and methyl octanoate. If it is assumed that component 1718 is a methyl 2-methylalkanoates ($C_9H_{18}O_2$, 158 Da), an $[M-59]^+$ ion at m/z 99 would be expected:

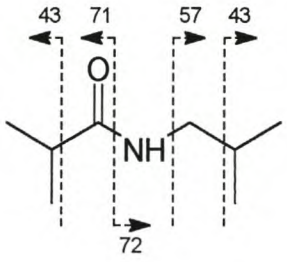


Although there was not an m/z 99 ion or a molecular ion present in the mass spectrum of this component, the presence of the m/z 59 ion could be ascribed to this component being a methyl ester. Since no methyl 2-methylalkanoates are commercially available, this route was not pursued any further. A computer search[13] gave 4-acetoxy-2-butanone (3-oxobutyl acetate) as a possible candidate, but retention-time testing with the commercial compound proved otherwise, owing to a too long retention time. No further work was done on this component.

The EI mass spectrum of component 1839 (Fig. 2.122) has the same general appearance as that of component 1718 (Fig. 2.121), although the relative abundance of the ions differ. The same general discussion concerning the possibility that component 1718 could be a 2-ethylalkanoic acid can be applied here except for the fact that component 1839 does have an m/z 60 ion in its mass spectrum. This could indicate that component 1839 is 2-ethyl-3-methylbutanoic acid. This acid was not available and could therefore not be retention-time tested. The m/z 60, 61 and 88 ions could indicate that the component is an ethyl ester. Component 1839 has a retention time between that of ethyl heptanoate and ethyl octanoate and could therefore be an ethyl ester of a branched octanoic acid. Ethyl esters of this type are not readily available and could therefore not be subjected to retention-time testing. The same general discussion of the possibility that component 1718 could be a methyl 2-methylalkanoate is also applicable to component 1839, with the difference that there is an m/z 99 $[M-59]^+$ ion present in the mass spectrum of this component.

This could indicate an ester of the mentioned type, but since these esters were not commercially available, retention-time comparisons could not be done. No further work was done on this component.

The m/z 143 ion in the EI mass spectrum of component 2119 (Fig. 2.123), which was assumed to be the molecular ion of this component, together with the ions at m/z 43 and m/z 71 (75-100%), were used as parameters in a computer search[13]. This returned *N*-isobutyl-isobutanamide as a result:



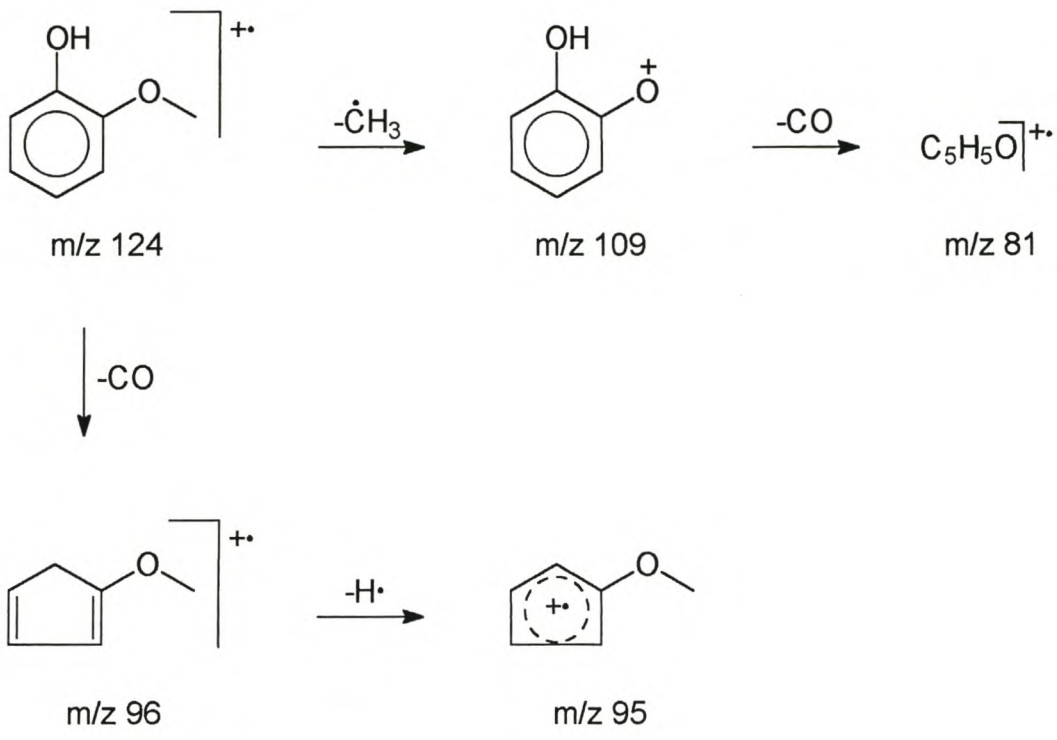
The following isobutanamides were synthesized and retention-time tested, with the results given below in increasing order of retention time:

Isobutanamide	Retention time
N-*tert*-butyl-isobutanamide	Too short
N,*N*-diethyl-isobutanamide	Too short
N-isopropyl-*N*-methyl-isobutanamide	Too short
N-propyl-*N*-methyl-isobutanamide	Too long
N-*sec*-butyl-isobutanamide	Too long
N-isobutyl-isobutanamide	Too long
N-butyl-isobutanamide	Too long

No further work was done on this component.

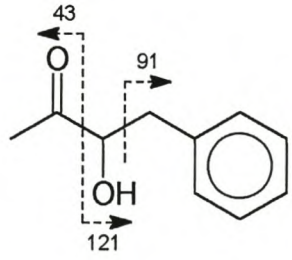
The only compound tested for component 2510 (Fig. 2.124) was cumic alcohol (4-isopropylbenzyl alcohol) (bp 249°C), after a computer search[13] suggested this alcohol. The retention time of cumic alcohol, however, is 10 minutes too long to be component 2510. No further work was done on this component.

The only two compounds tested as possible candidates for component 2707 (Fig. 2.125) were 2- and 4-methoxyphenol. The ions at m/z 124, 109, 96, 95, and 81 are formed as follows, using 2-methoxyphenol as example:



The retention times of 2-methoxyphenol (guaiacol) (bp 202°C) and 4-methoxyphenol (bp 243°C) were, respectively, shorter and longer than that of component 2707. No further work was done on this component.

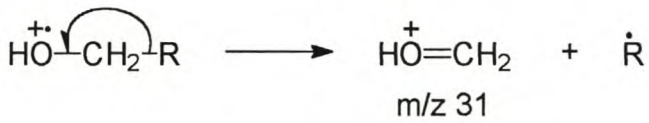
A computer search indicated that component 3449 (Fig. 2.126) could possible be 3-hydroxy-4-phenyl-2-butanone:



This compound is not commercially available and therefore retention-time testing could not be done. No further work was done on this component.

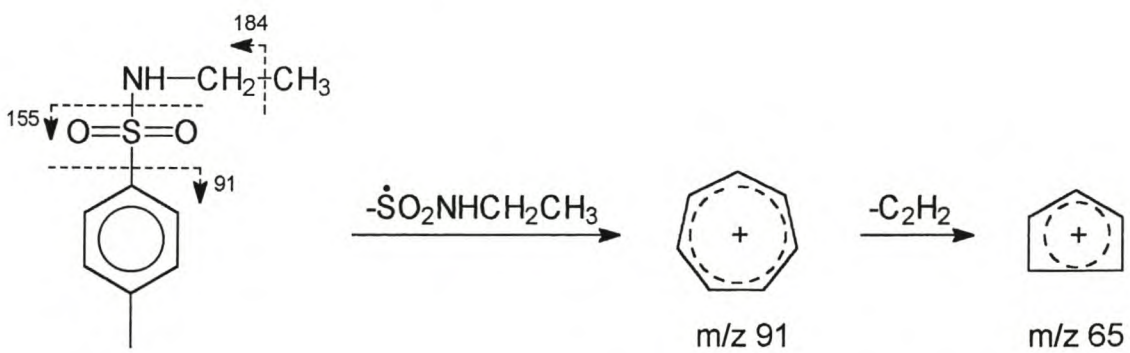
The EI mass spectrum of component 5028 (Fig. 2.127) has the same general appearance as that of the 1-alkanols already identified in the interdigital secretion (see § 2.2.4). This component has a retention time between that of 1-hexadecanol and 1-heptadecanol and could therefore be a branched 1-heptadecanol. The

absence from the spectrum of an m/z 31 ion, which is formed by the mechanism shown below, was taken as evidence that this component is not a 1-alkanol:



A computer search[13] selected formates as possible candidate compounds and after injecting a series of synthetic unbranched formates (tetradecyl formate through docosyl formate), it was apparent that hexadecyl formate had a retention time of about 50 seconds too long to be component 5028. No further work was done on this component.

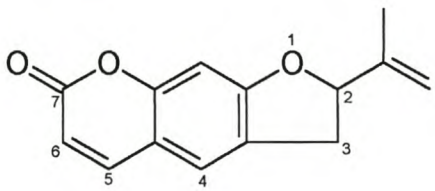
In addition to ions at m/z 184 and m/z 91 that can be ascribed to the loss of a methyl group from the molecular ion and a tropylium ion, respectively, the EI mass spectrum of component 5253 (Fig. 2.128) contains an ion at m/z 199, assumed to be the molecular ion of the compound. The loss of 64 atomic mass units between m/z 155 and m/z 91 was ascribed to the elimination of SO_2. These assumptions, together with the relative abundance of the ions, were used in a computer search, which yielded only one result, namely *N*-ethyl-*p*-toluenesulfonamide, which had a mass spectrum similar to that of component 5253:



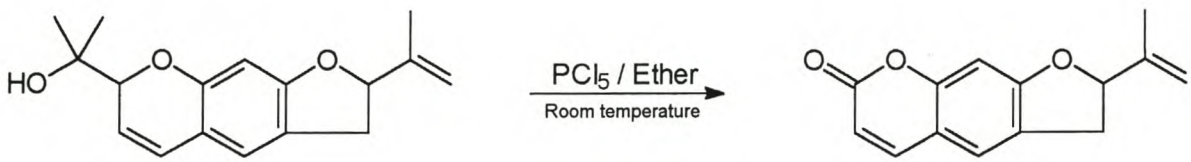
This compound was not available for retention-time testing. No further work was done on this compound.

Component 5431 (Fig. 2.129) in the TIC was identified as ammarin (2,3-dihydro-2-(1-methylethenyl)-7*H*-furo[3,2-g][1]benzopyran-7-one), a coumarin, purely on the basis of a computer search[13], but since this compound was not

commercially available, it could not be confirmed in the usual manner by co-injection with the natural extract:



Ammarin has been isolated from *Ammi majus*[93] and *Scaevola frutescens*[94] and has been prepared from marmesin[93] as follows:



Isolated ammarin from these plants has been assigned the 2'(*R*)-configuration[95], whereas the corresponding (*S*)-diastereoisomer (isoangenomalin or (*S*)-*exo*-anhydromarmesin), synthesized by Ishi and Ishikawa[95], was extracted from only *S. lobelia*[96,97].

2.2.33 Impurities

Components 245 (Fig. 2.130) and 258 (Fig. 2.131) were identified as chloroform and 2-chloro-2-methylbutane, respectively. These components are known to be solvent impurities.

Component 266 (Fig. 2.132) has the familiar benzene mass spectrum and co-injection of synthetic benzene with the natural secretion confirmed that component 266 is benzene. Since benzene is such a common solvent, it was assumed that this component was an artefact in the secretion.

2.3 Conclusion

The one hundred and ten components identified in the interdigital secretion of the black wildebeest in this study are listed in Table 2.1.

Table 2.1: Compounds identified in the interdigital secretion of the black wildebeest, *Connochaetes gnou*

§	Compound	EI mass spectrum Fig.	Synthesis §	Male Fig.	Female Fig.
2.2.1	Octane	2.3		412	401
2.2.1	Decane	2.4		1070	1057
2.2.1	2-Methylheptane	2.5	3.4.1	343	334
2.2.2	1-Heptyne	2.6		330	321
2.2.3	Toluene	2.7		450	440
2.2.3	Ethylbenzene	2.8		723	711
2.2.3	*p*-Xylene	2.9		751	738
2.2.4	1-Dodecanol	2.10		3553	3544
2.2.4	1-Tridecanol	2.11		3929	3920
2.2.4	1-Pentadecanol	2.12		4632	4623
2.2.4	1-Hexadecanol	2.13		4962	4954
2.2.5	3-Methyl-3-buten-2-ol	2.14	3.4.2	398	387
2.2.5	3-Methyl-3-buten-1-ol	2.15		525	511
2.2.6	3,3-Dimethylcyclohexanol	2.16	3.4.3	1575	1563
2.2.6	Verbenol	2.17	3.4.4	2207	2195
2.2.6	1-Borneol	2.18		2334	2323
2.2.7	Phenol	2.19		2058	2045
2.2.7	*p*-Cresol	2.20		2432	2420
2.2.7	*m*-Cresol	2.21		2440	2428
2.2.7	4-Ethylphenol	2.22		2815	2803
2.2.7	3-Ethylphenol	2.23		2825	2813
2.2.7	3-Propylphenol	2.24		3181	3170
2.2.7	Benzyl alcohol	2.25		1992	1979
2.2.7	1-Phenylethanol	2.26		2028	2014
2.2.7	2-Phenylethanol	2.27		2299	2287
2.2.7	1-Phenyl-1,2-ethanediol	2.28		3485	3476
2.2.8	(*R*,*R*)- and/or (*S*,*S*)-2,3-Butanediol	2.29		949	933
2.2.8	*meso*-2,3-Butanediol	2.30		988	971
2.2.9	Di(ethylene glycol)monoethyl ether	2.31		1664	1651
2.2.9	Tri(ethylene glycol)	2.32		3081	3076
2.2.9	Tetra(ethylene glycol)	2.33		4180	4167
2.2.10	3-Methylbutanal	2.34		298	289
2.2.10	Nonanal	2.35		*	1909
2.2.10	Tridecanal	2.36		3640	3629
2.2.10	Tetradecanal	2.37		4021	4010
2.2.11	2-Methyl-2-propenal	2.38	3.4.5	204	197
2.2.12	Benzaldehyde	2.39		1413	1400
2.2.12	Vanillin	2.40		3769	3758
2.2.12	4-Hydroxybenzaldehyde	2.41		4220	4209
2.2.12	2-Hydroxybenzaldehyde	2.42		1789	1775
2.2.13	3-Methyl-2-butanone	2.43		314	306
2.2.13	4-Heptanone	2.44		889	875

2.2.13	2-Heptanone	2.45		*	983
2.2.13	6-Methyl-2-heptanone	2.46	3.4.6	1269	1255
2.2.13	2-Octanone	2.47		1436	1422
2.2.13	3-Octanone	2.48		1169	1155
2.2.13	4-Nonanone	2.49		1766	1753
2.2.14	7-Octen-2-one	2.50		1452	1438
2.2.14	*trans*-6-Methyl-3,5-heptadien-2-one	2.51		2131	2118
2.2.15	Isophorone	2.52		2264	2251
2.2.15	Verbenone	2.53		2672	2660
2.2.16	2,3-Butanedione	2.54		238	230
2.2.17	Acetophenone	2.55		1939	1927
2.2.17	4-Hydroxyacetophenone	2.56		4456	4445
2.2.18	3-Hydroxy-2-butanone	2.57		536	519
2.2.18	3-Hydroxy-3-methyl-2-butanone	2.58	3.4.10	583	571
2.2.18	3-Hydroxy-2-pentanone	2.59	3.4.11	805	792
2.2.18	2-Hydroxy-3-pentanone	2.60	3.4.12	842	829
2.2.19	4-Hydroxy-3,6,6-trimethyl-2-cyclohexen-1-one	2.61	3.4.13	3106	3095
2.2.20	Acetic acid	2.62		367	353
2.2.20	Propanoic acid	2.63		667	644
2.2.20	Butanoic acid	2.64		957	943
2.2.20	Pentanoic acid	2.65		1375	1363
2.2.20	Hexanoic acid	2.66		1807	1795
2.2.20	Nonanoic acid	2.67		3052	3048
2.2.20	Decanoic acid	2.68		3432	3425
2.2.20	Dodecanoic acid	2.69		4154	4146
2.2.20	Tridecanoic acid	2.70		4494	4485
2.2.20	Tetradecanoic acid	2.71		4825	4821
2.2.20	Pentadecanoic acid	2.72		5137	5130
2.2.20	Hexadecanoic acid	2.73		5458	5460
2.2.20	Heptadecanoic acid	2.74		5736	5730
2.2.20	Octadecanoic acid	2.75		6028	6027
2.2.20	2-Methylpropanoic acid	2.76		829	820
2.2.20	3-Methylbutanoic acid	2.77		1181	1166
2.2.20	2-Methylbutanoic acid	2.78		1206	1190
2.2.20	2-Methylpentanoic acid	2.79	3.4.14	1588	1577
2.2.20	2,3-Dimethylbutanoic acid	2.80	3.4.15	1510	1502
2.2.20	2-Ethylbutanoic acid	2.81	3.4.16	1559	1549
2.2.21	(*Z*)-9-Octadecenoic acid	2.82		5986	5981
2.2.22	Cyclohexanecarboxylic acid	2.83		2486	2492
2.2.23	Benzoic acid	2.84		2912	2917
2.2.23	Phenylacetic acid	2.85		3260	3253
2.2.24	Phthalic anhydride	2.86		3471	3459
2.2.25	Methyl pentanoate	2.87		655	642
2.2.25	Methyl hexanoate	2.88		1030	1016
2.2.26	Ethyl acetate	2.89		234	**
2.2.26	Ethyl butanoate	2.90		576	564
2.2.26	Ethyl pentanoate	2.91		924	910
2.2.26	2-Phenylethyl acetate	2.92		2728	2717
2.2.26	1-Phenylethyl 2-methylpropanoate	2.93	3.4.17	2908	2896
2.2.26	2-Phenylethyl 2-methylpropanoate	2.94	3.4.18	3256	3246
2.2.26	2-Phenylethyl hexadecanoate	2.95	3.4.18	7202	7194
2.2.26	Triethyl citrate	2.96		4572	4561
2.2.27	3-Methyl-3-butenyl acetate	2.97	3.4.19	902	888
2.2.28	Dimethyl phthalate	2.98	3.4.20	3797	3786
2.2.28	Methyl 3-hydroxybenzoate	2.99		4385	4374
2.2.29	2-Piperidone	2.100		2982	2984
2.2.29	6-Hexanelactam	2.101	3.4.21	3227	3226

2.2.30	Cholesterol	2.102		8805	8801
2.2.31	Tetrahydrofuran	2.103		*	232
2.2.31	(*Z*,*Z*)-Dipropenyl ether	2.104	3.4.22	307	298
2.2.31	1-Bromohexyne	2.105		853	840
2.2.31	Benzyl chloride	2.106(a)(b)		1541	1528
2.2.31	Dimethyl sulfone	2.107		1922	1909
2.2.31	Pummerer's ketone	2.108	3.4.23	5177	5164
2.2.31	1*H*-Indole-3-carboxaldehyde	2.109		5877	5866
2.2.31	4-Octadecanolide	2.110		6593	6585
2.2.32	Unidentified component	2.111		435	423
2.2.32	Unidentified component	2.112	3.4.24	603	591
2.2.32	Unidentified component	2.113	3.4.25	757	742
2.2.32	Unidentified component	2.114	3.4.26	793	780
2.2.32	Unidentified component	2.115		888	874
2.2.32	Unidentified component	2.116		998	-
2.2.32	Unidentified component	2.117	3.4.27	1138	1125
2.2.32	Unidentified component	2.118		1240	1226
2.2.32	Unidentified component	2.119		1315	1301
2.2.32	Unidentified component	2.120	3.4.28	1386	1375
2.2.32	Unidentified component	2.121		1718	1705
2.2.32	Unidentified component	2.122		1839	1831
2.2.32	Unidentified component	2.123		2119	2106
2.2.32	Unidentified component	2.124		2510	2498
2.2.32	Unidentified component	2.125		2707	2695
2.2.32	Unidentified component	2.126		3449	3438
2.2.32	Unidentified component	2.127		5028	5018
2.2.32	Unidentified component	2.128		5253	5242
2.2.32	Unidentified component	2.129		5431	5420
2.2.33	Chloroform	2.130		245	238
2.2.33	2-Chloro-2-methylbutane	2.131		258	250
2.2.33	Benzene	2.132		266	258

* Compound not found in the male black wildebeest interdigital secretion

** Compound not found in the female black wildebeest interdigital secretion

This study has not only shown that the black wildebeest interdigital secretion contains many components but that it also contains an astonishing variety of different compound types. Some of the components identified were under suspicion of being artifacts, for example phthalic anhydride (component 3471), dimethyl phthalate (component 3797), tetrahydrofuran (component 232), 1-bromohexyne (component 853), and benzyl chloride (component 1541), but since all of these compounds were present in both the male and female secretions, except for tetrahydrofuran which was only found in the female secretion, it must be accepted that they are part of the natural secretion. However, it does seem strange that these compounds are present in the extract of the secretion. If the precautions taken to avoid contamination in the laboratory are taken into account, it has to be accepted that these compounds are present in the secretion or that they were introduced as

artefacts into the interdigital cavity of at least one male and one female in the group of seven animals from which material was collected.

Only small qualitative and quantitative differences were found between the male and female interdigtal secretions.

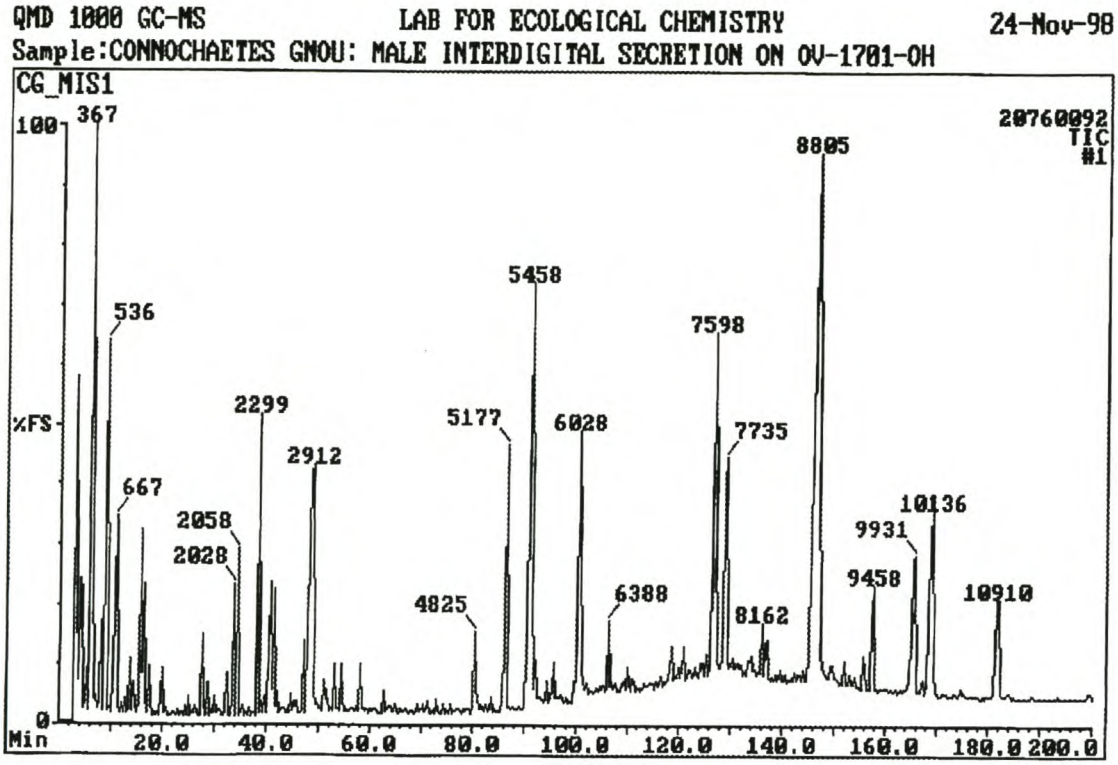


Fig. 2.1: TIC of the interdigital secretion of the male black wildebeest

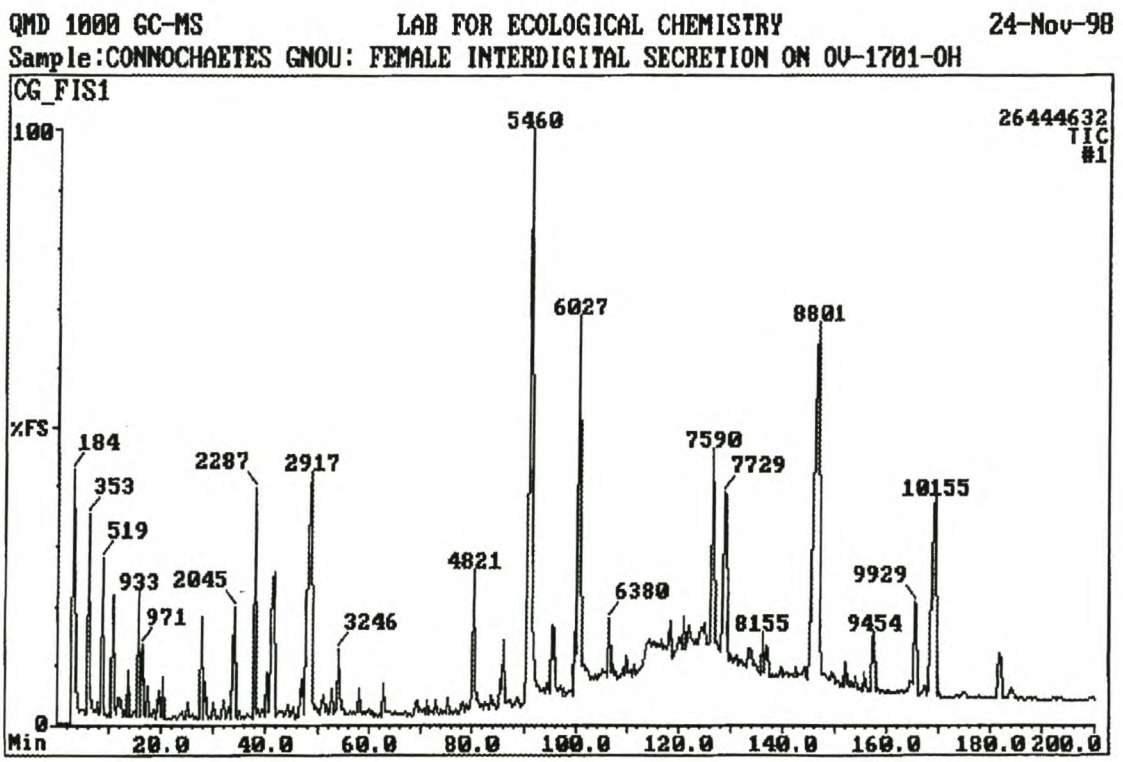


Fig. 2.2: TIC of the interdigital secretion of the female black wildebeest

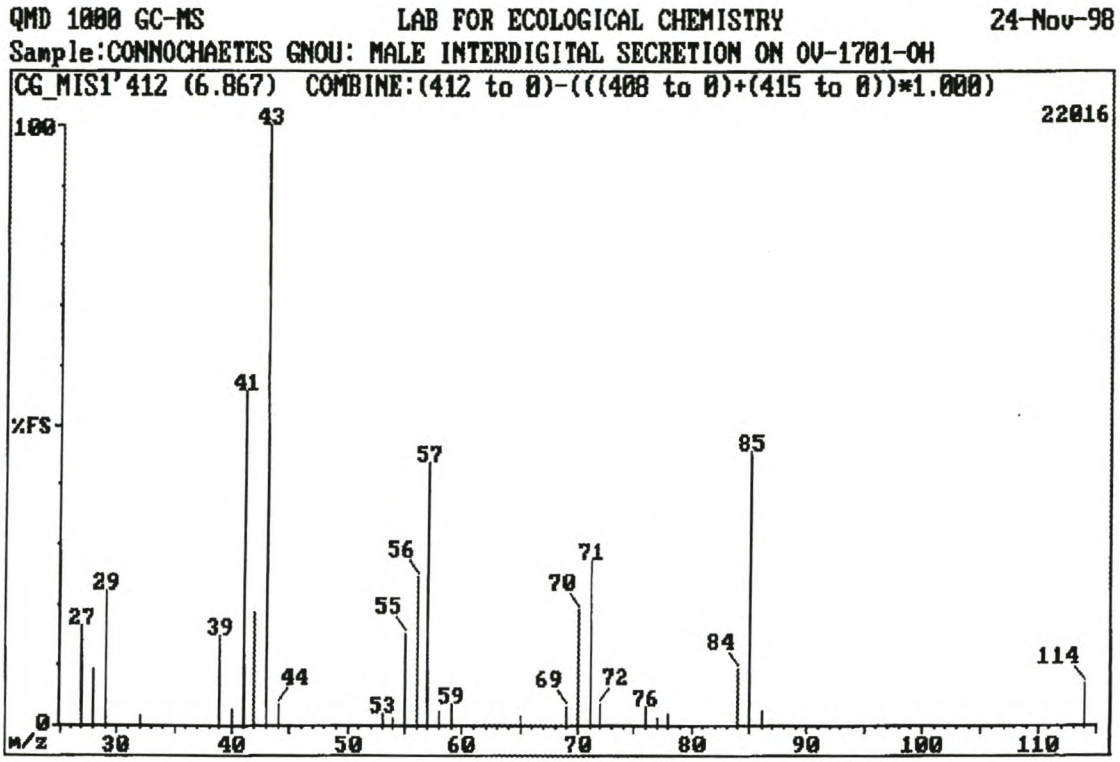


Fig. 2.3: EI mass spectrum of component 412 (octane)

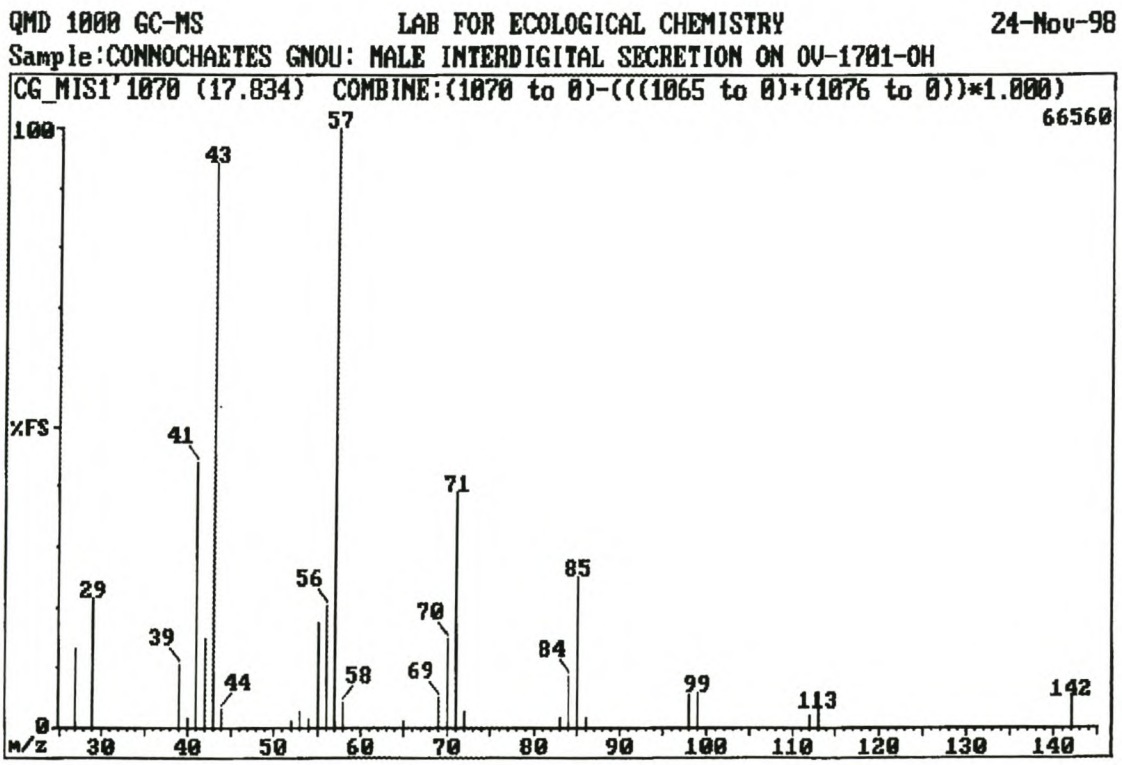


Fig. 2.4: EI mass spectrum of component 1070 (decane)

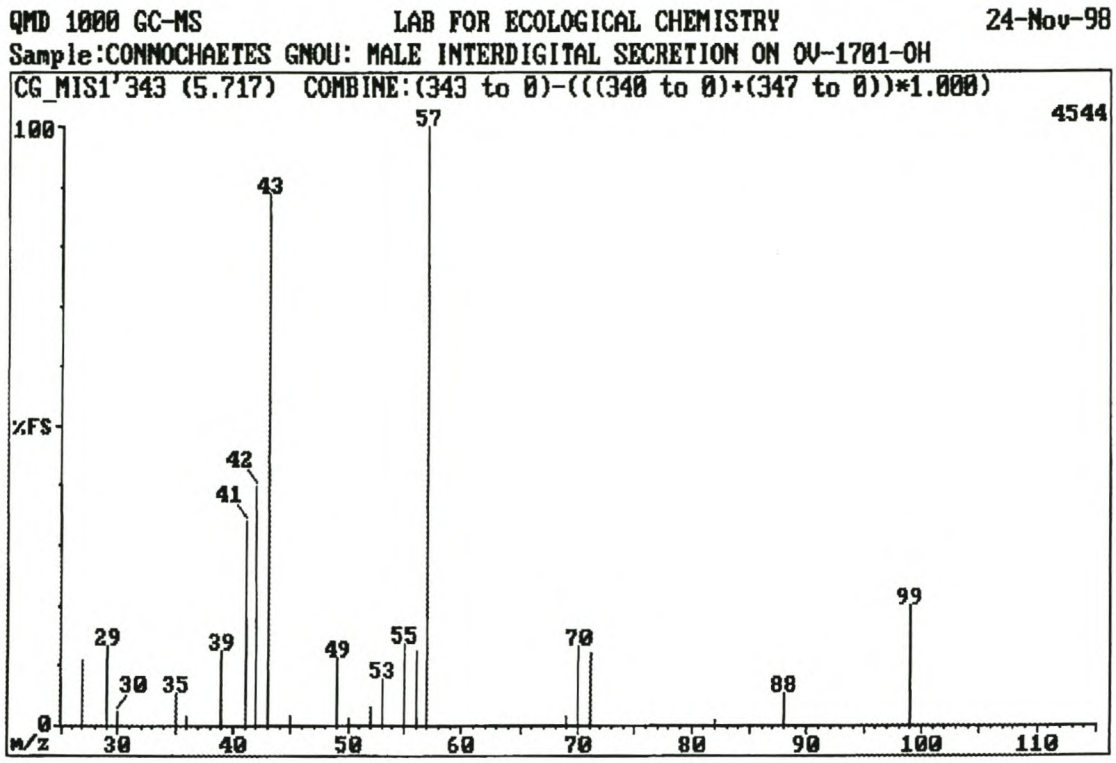


Fig. 2.5: EI mass spectrum of component 343 (2-methylheptane)

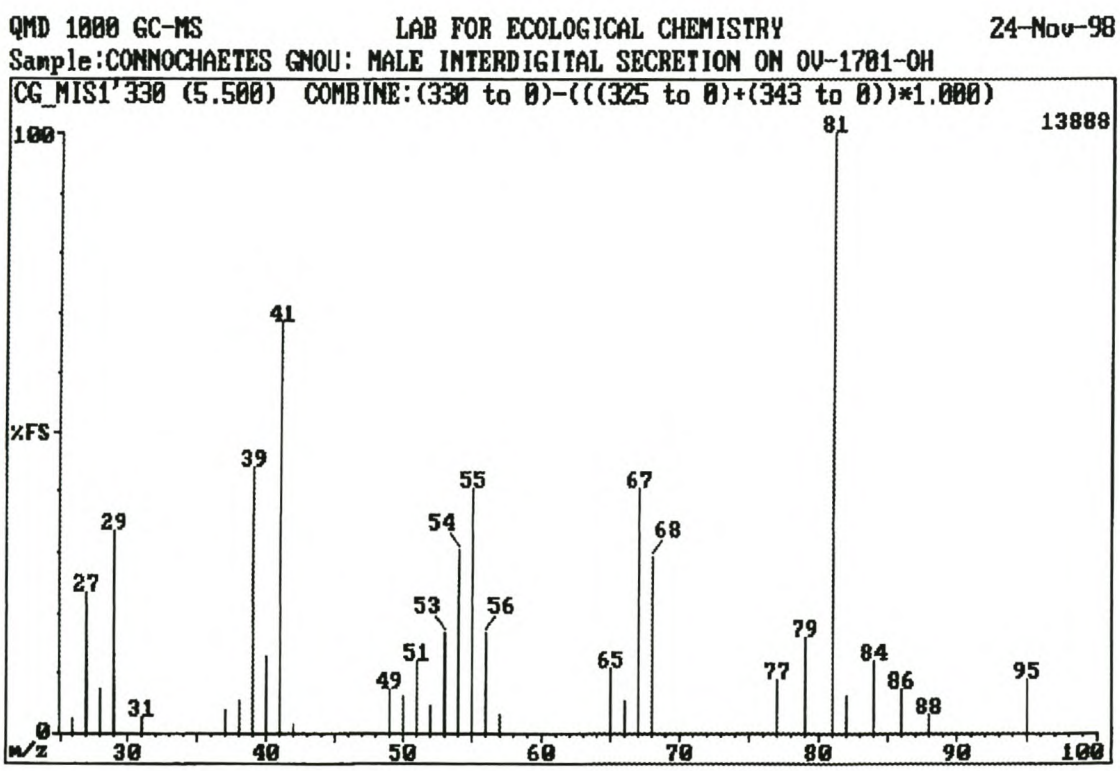


Fig. 2.6: EI mass spectrum of component 330 (1-heptyne)

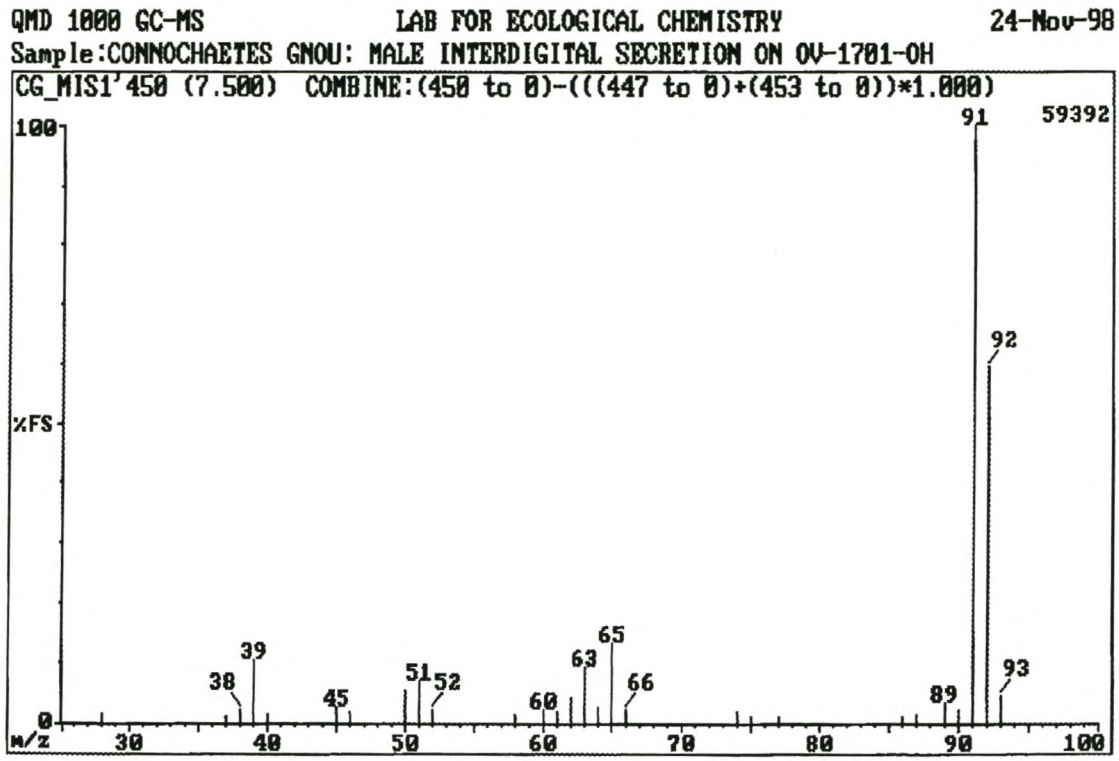


Fig. 2.7: EI mass spectrum of component 450 (toluene)

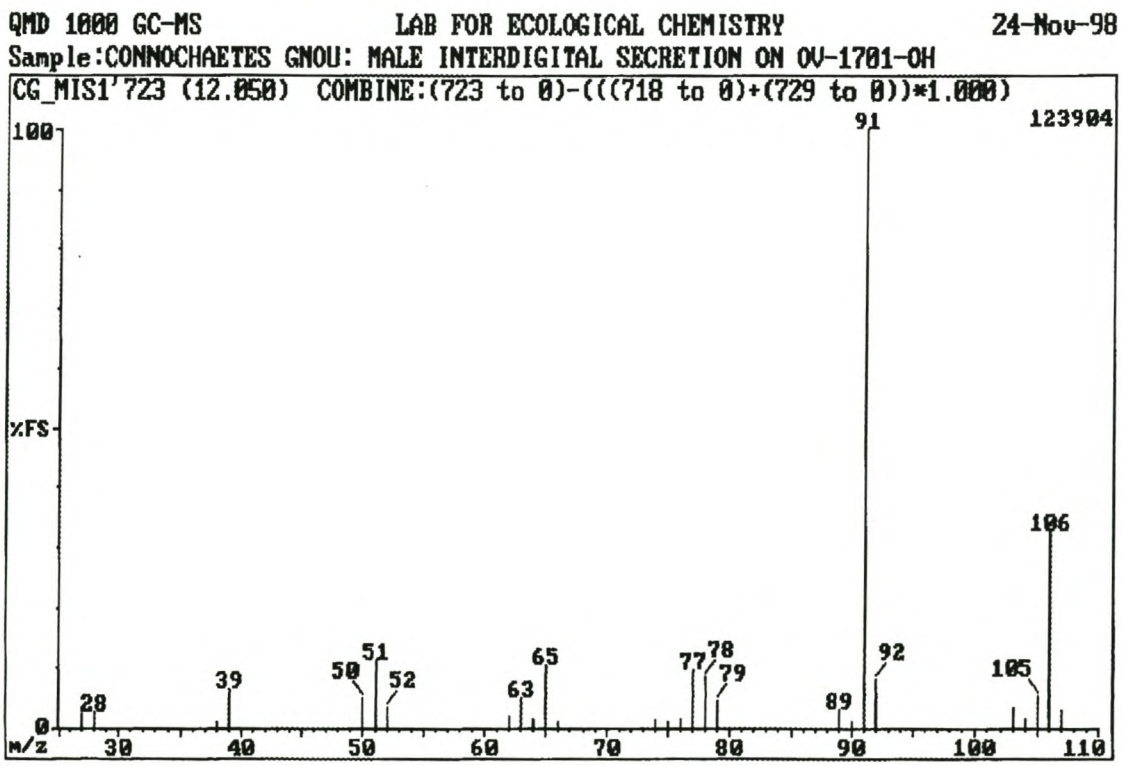


Fig. 2.8: EI mass spectrum of component 723 (ethylbenzene)

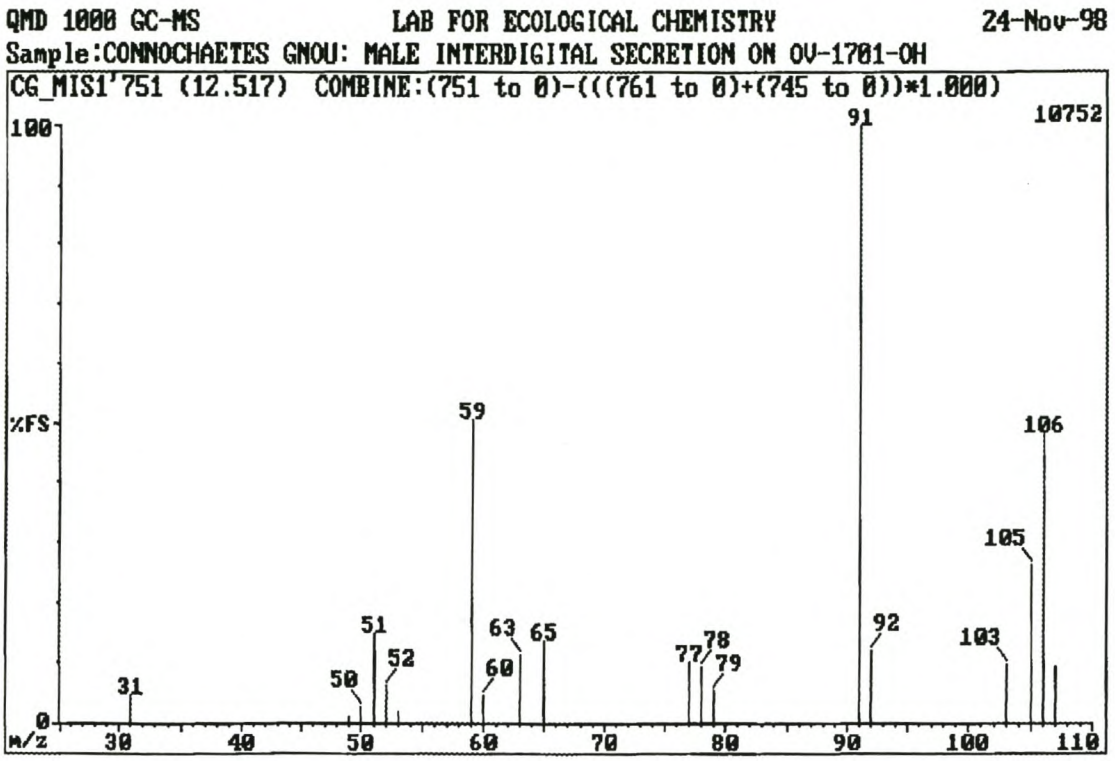


Fig. 2.9: EI mass spectrum of component 751 (*p*-xylene)

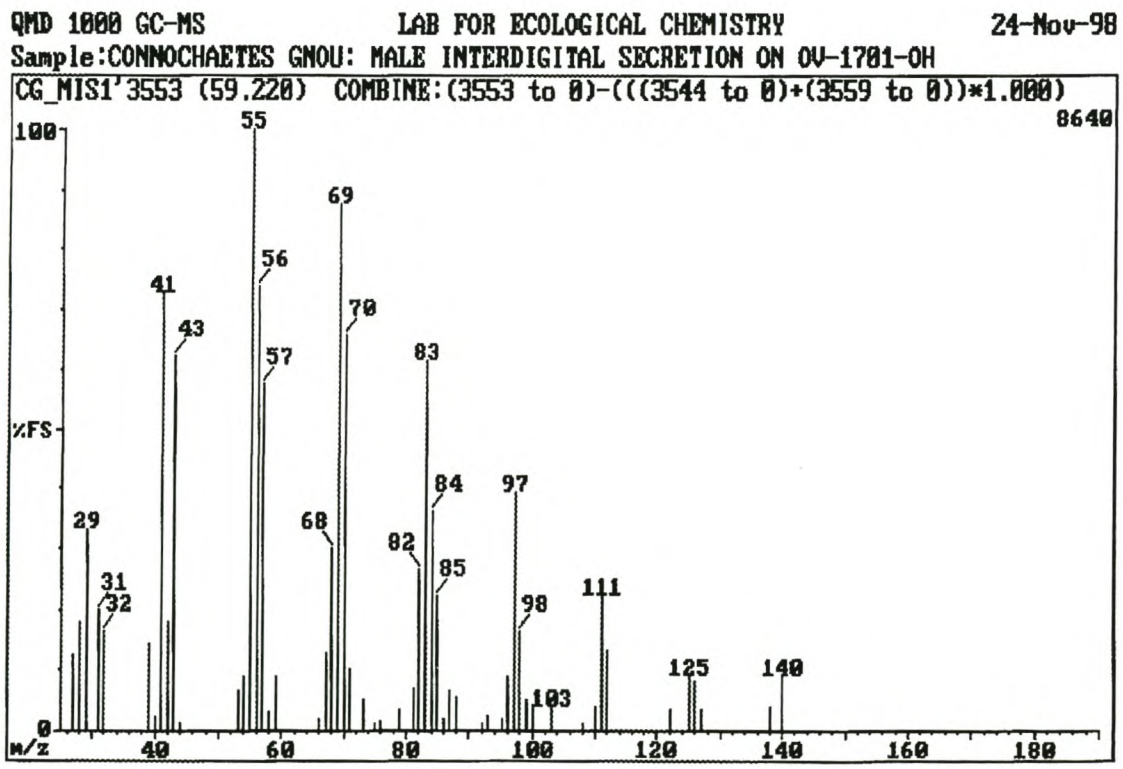


Fig. 2.10: EI mass spectrum of component 3553 (1-dodecanol)

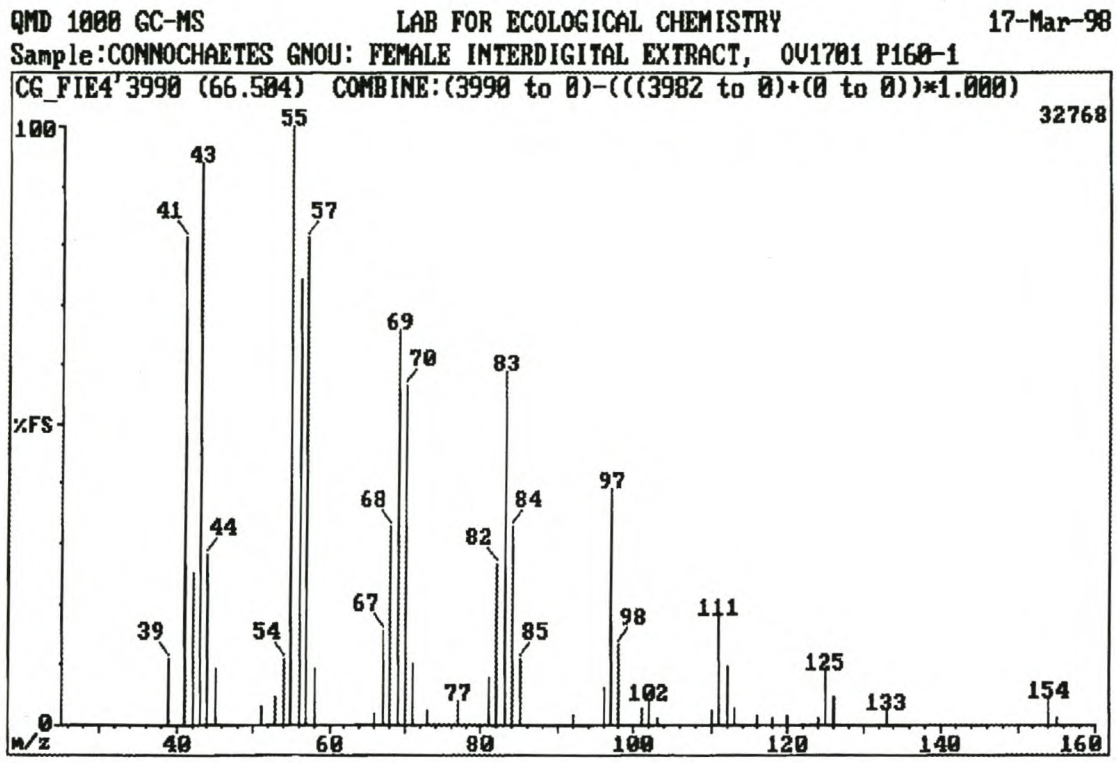


Fig. 2.11: EI mass spectrum of component 3929 (1-tridecanol)

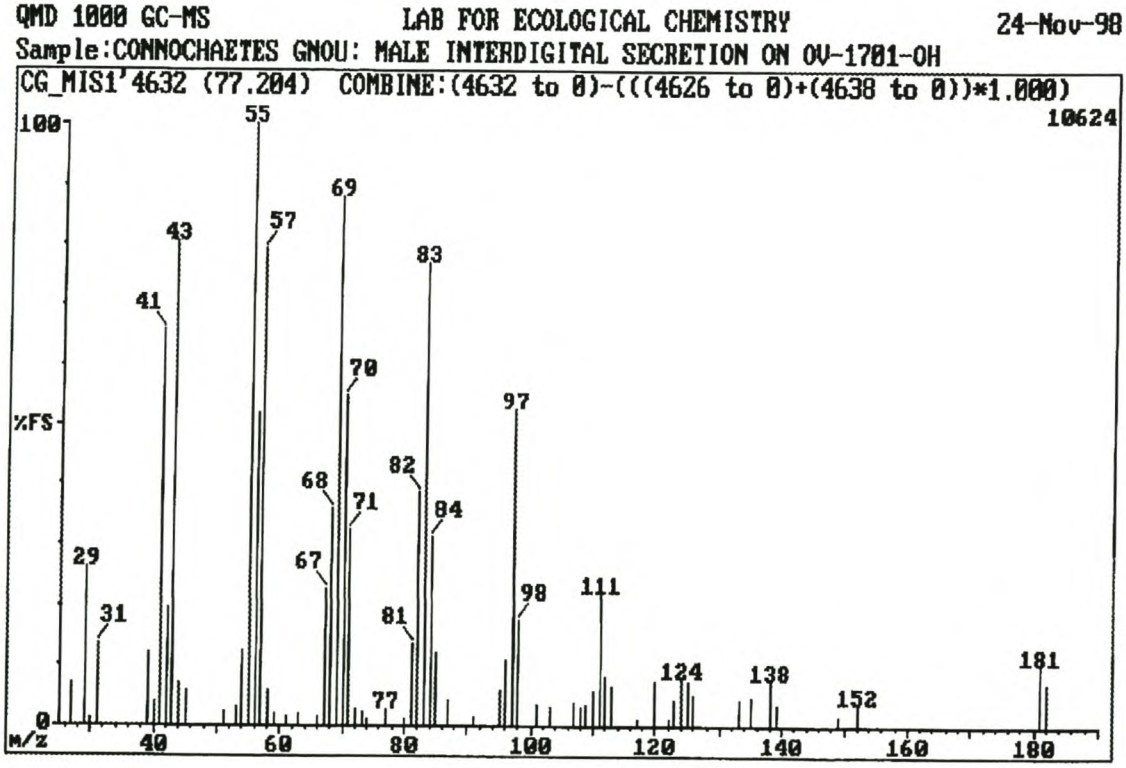


Fig. 2.12: EI mass spectrum of component 4632 (1-pentadecanol)

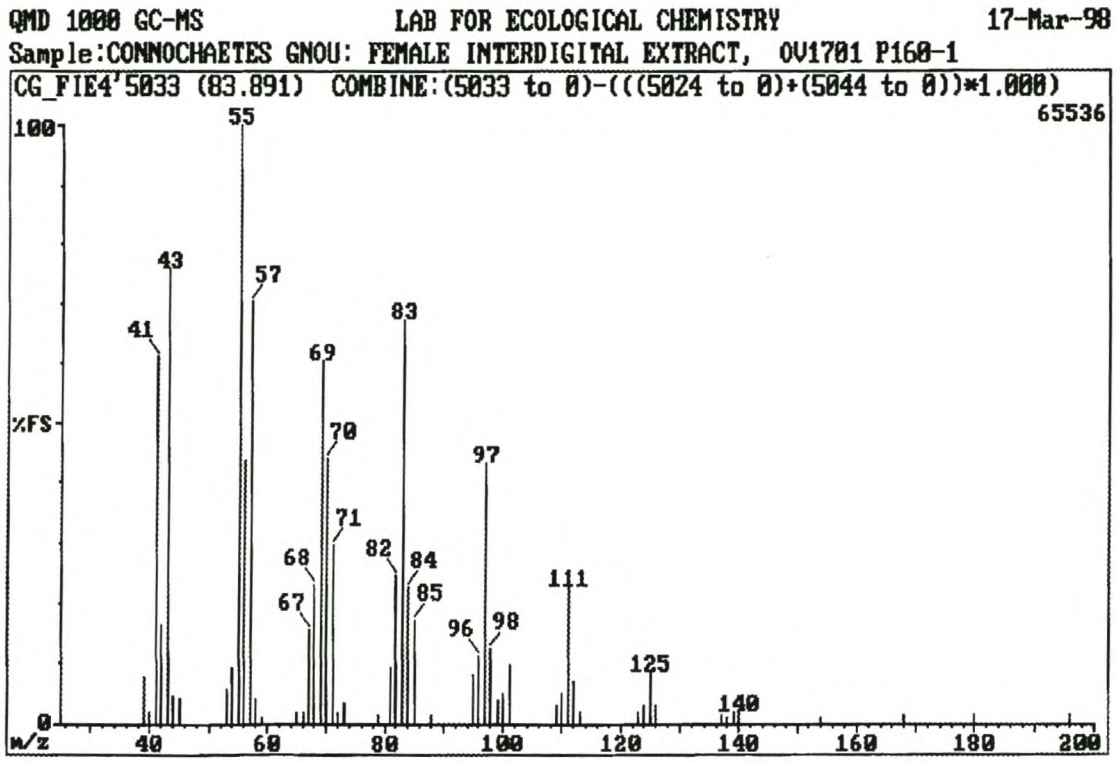


Fig. 2.13: EI mass spectrum of component 4962 (1-hexadecanol)

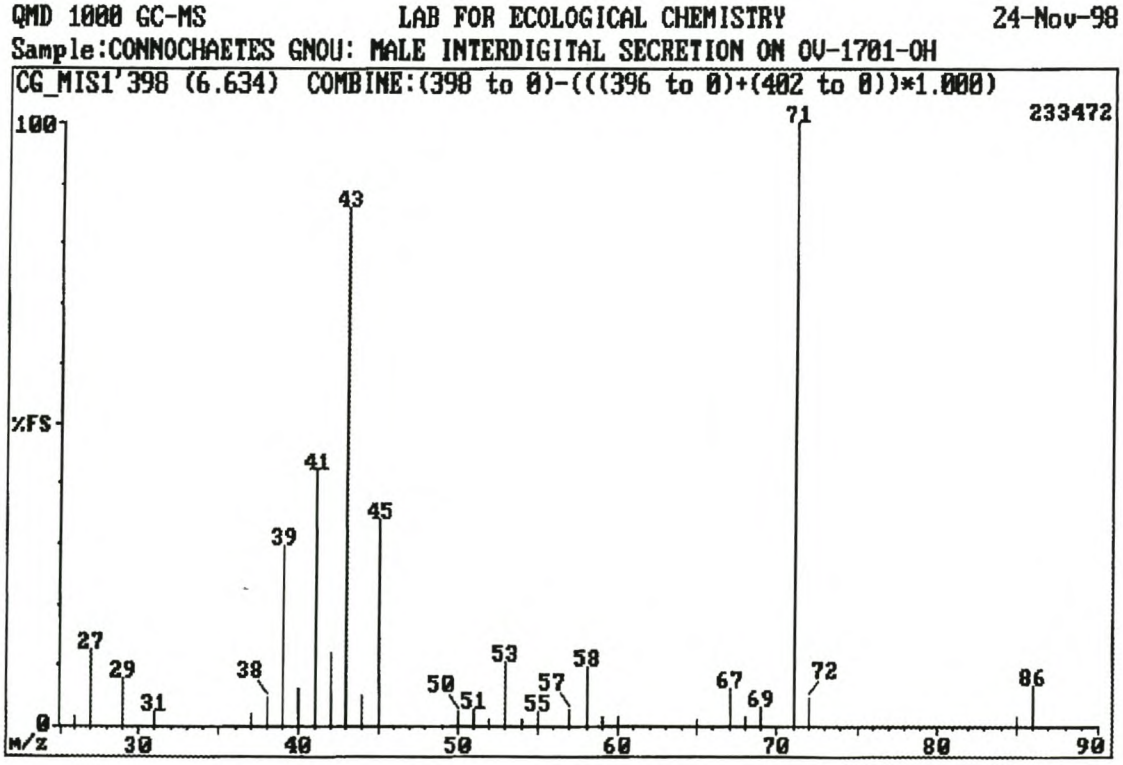


Fig. 2.14: EI mass spectrum of component 398 (3-methyl-3-buten-2-ol)

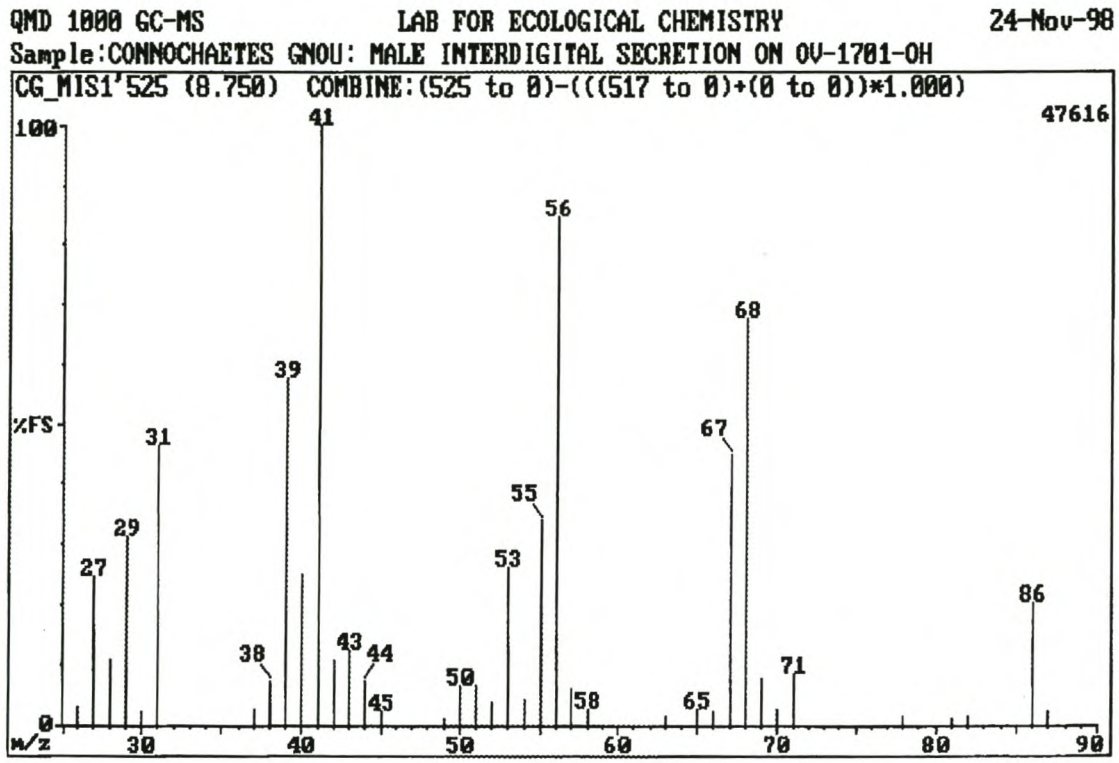


Fig. 2.15: EI mass spectrum of component 525 (3-methyl-3-buten-1-ol)

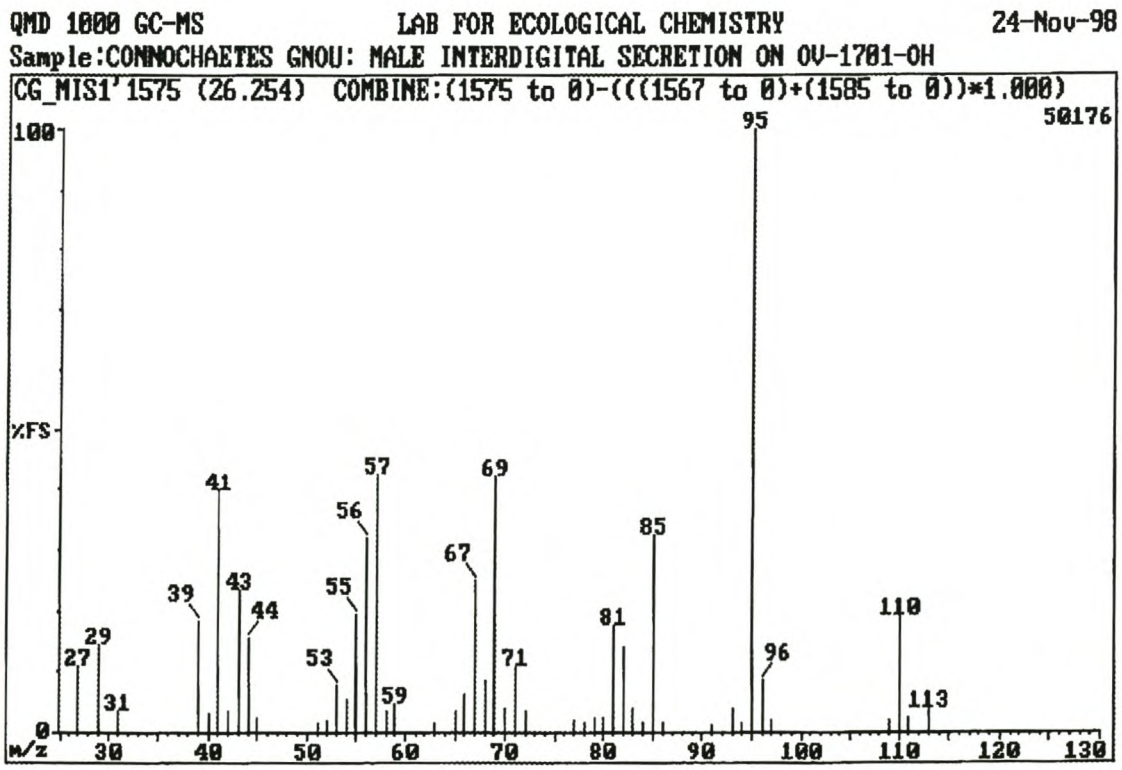


Fig. 2.16: EI mass spectrum of component 1575 (3,3-dimethylcyclohexanol)

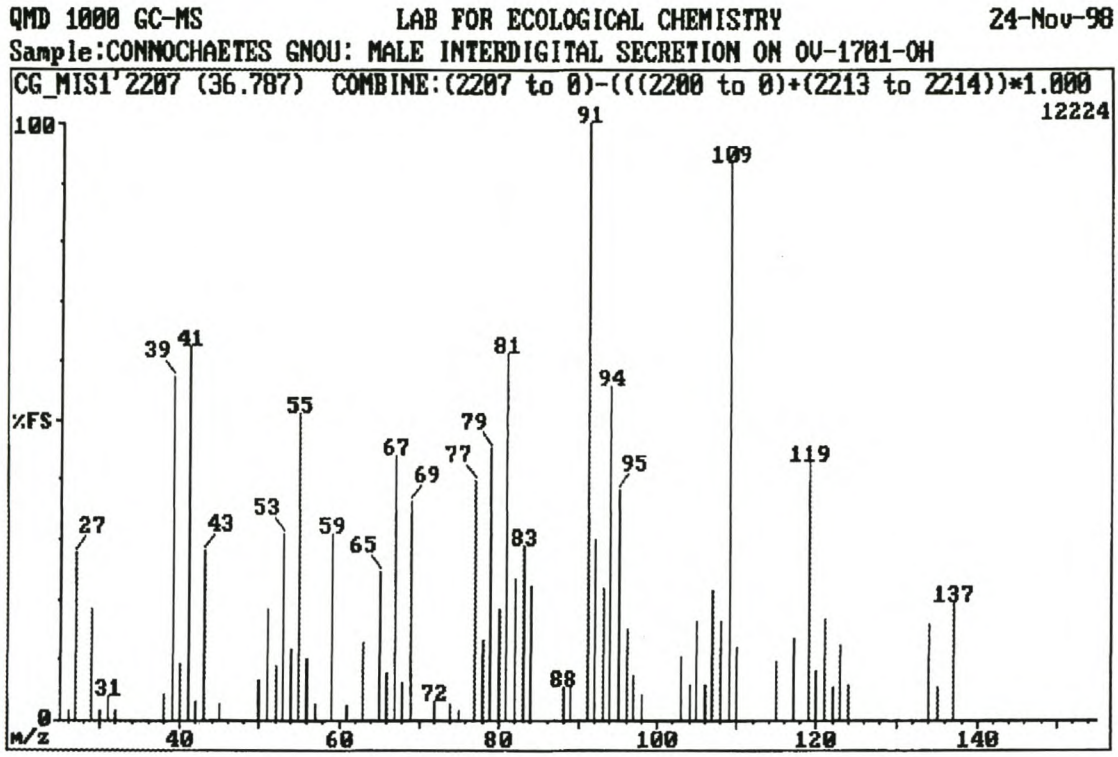


Fig. 2.17: EI mass spectrum of component 2207 (verbenol)

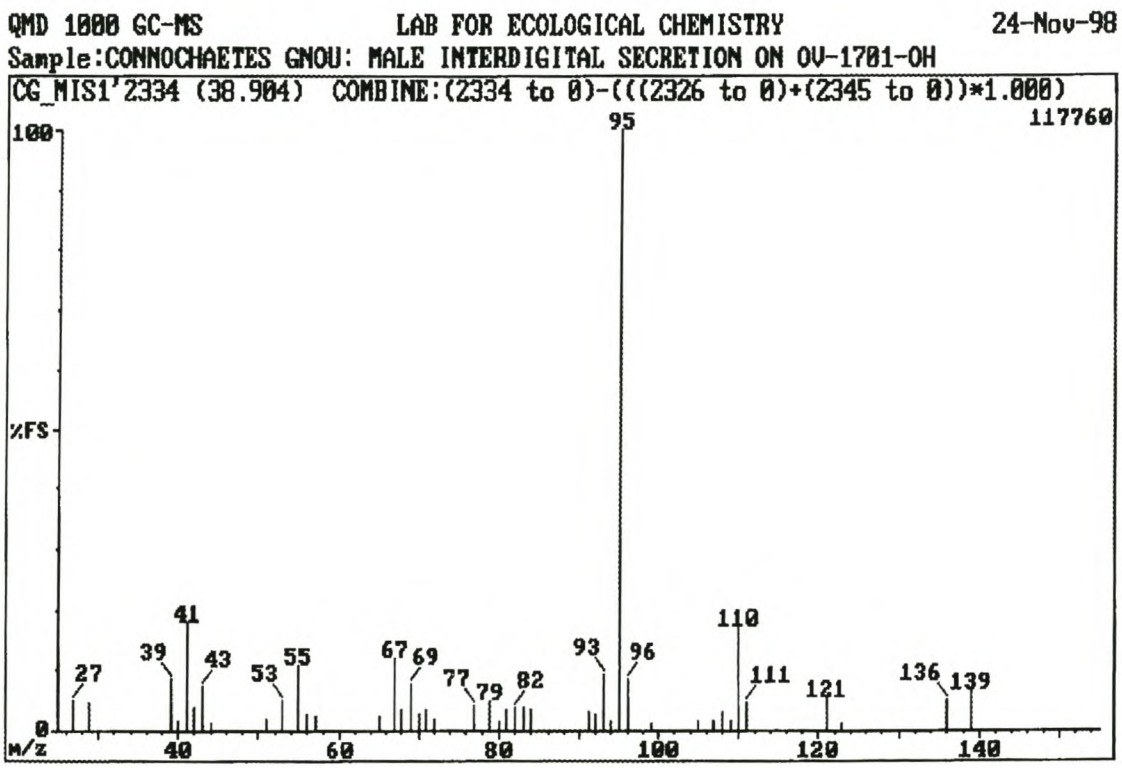


Fig. 2.18: EI mass spectrum of component 2334 (1-borneol)

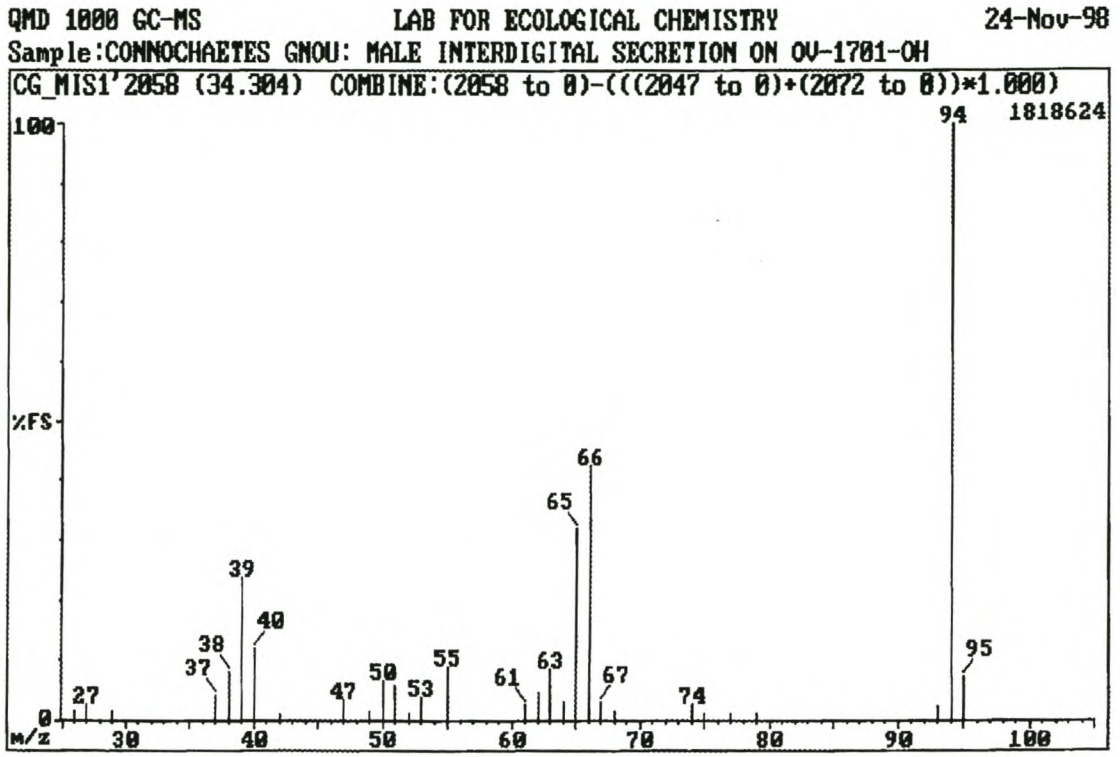


Fig. 2.19: EI mass spectrum of component 2058 (phenol)

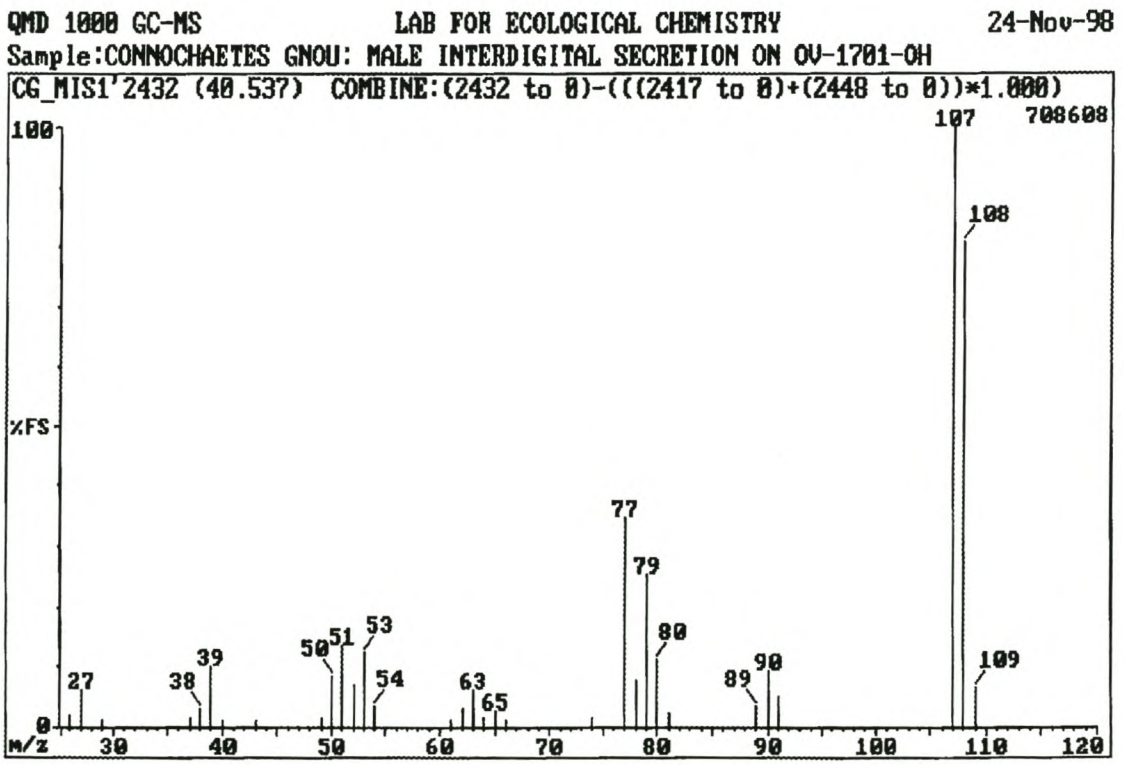


Fig. 2.20: EI mass spectrum of component 2432 (*p*-cresol)

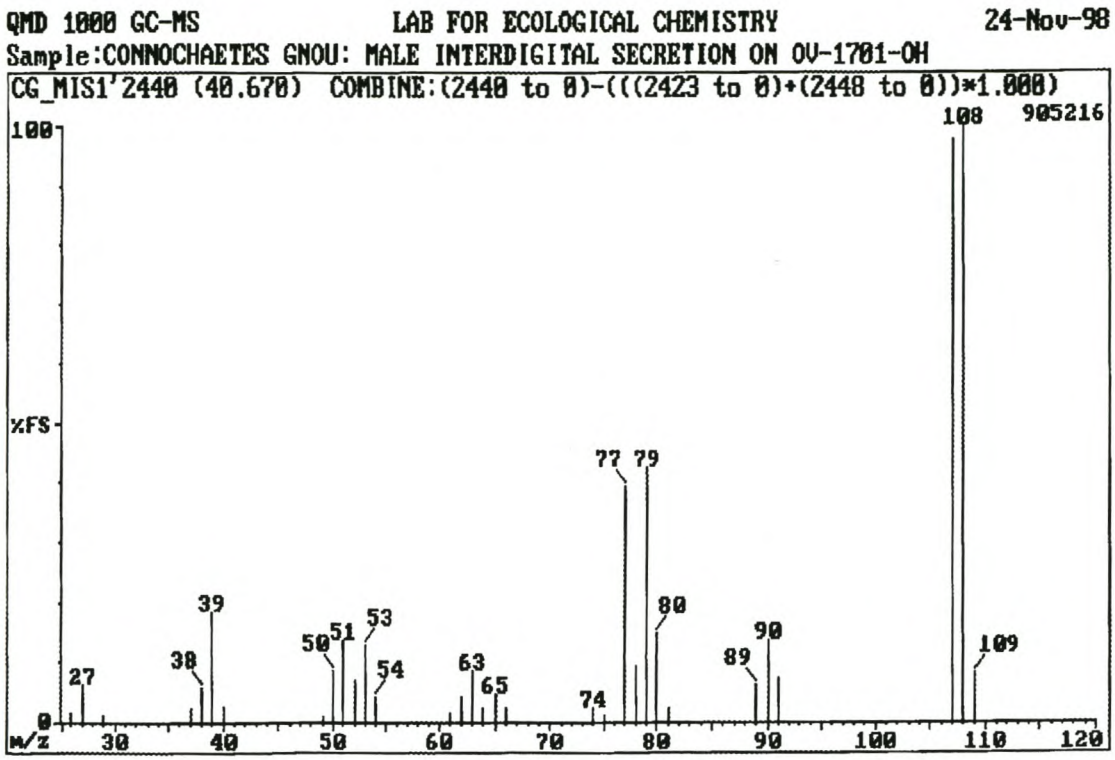


Fig. 2.21: EI mass spectrum of component 2440 (*m*-cresol)

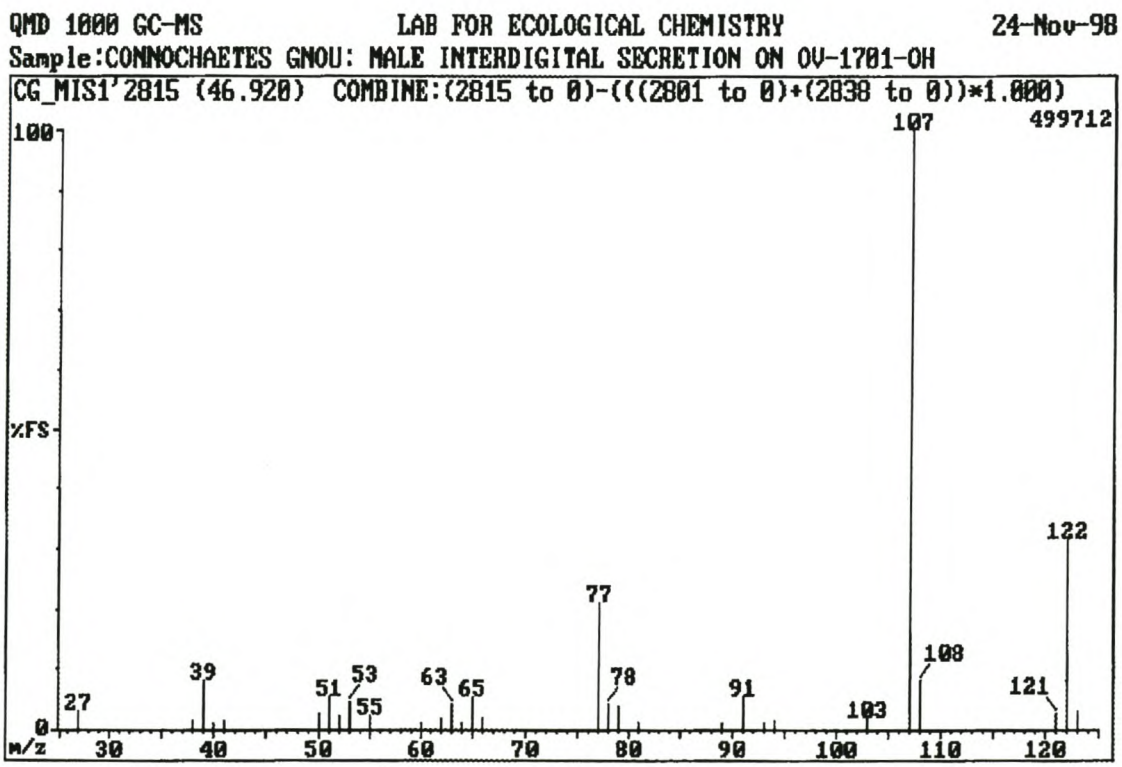


Fig. 2.22: EI mass spectrum of component 2815 (4-ethylphenol)

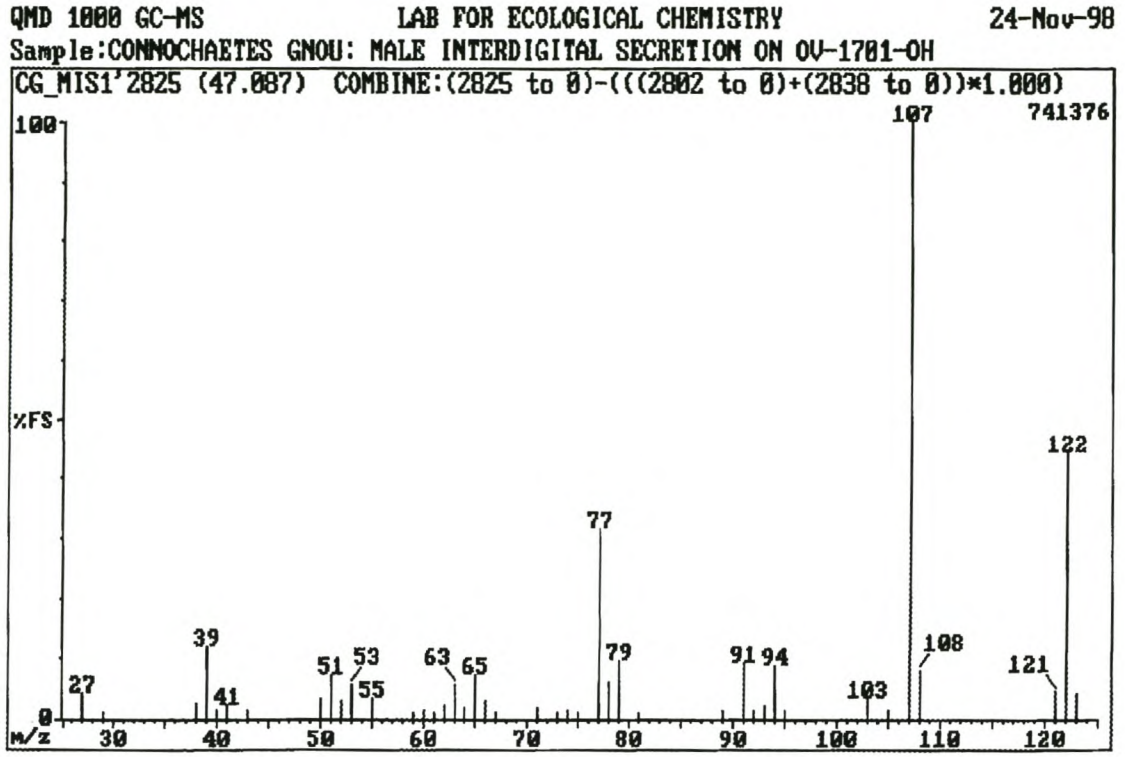


Fig. 2.23: EI mass spectrum of component 2825 (3-ethylphenol)

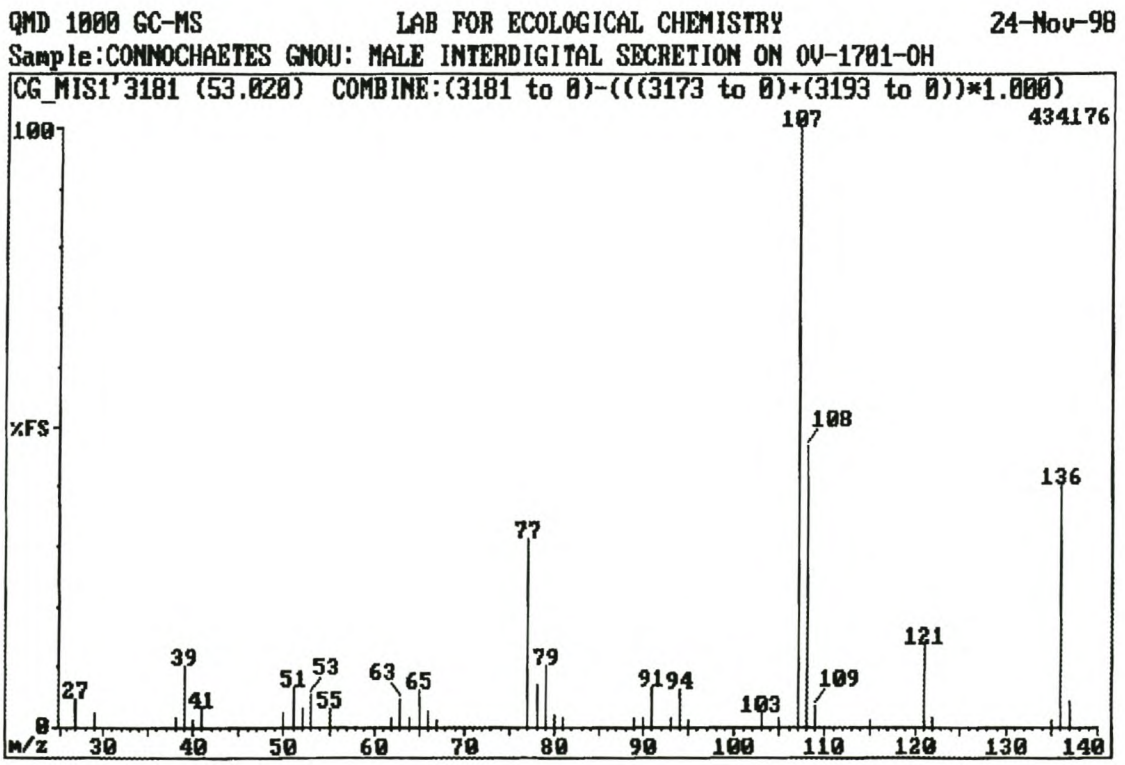


Fig. 2.24: EI mass spectrum of component 3181 (3-propylphenol)

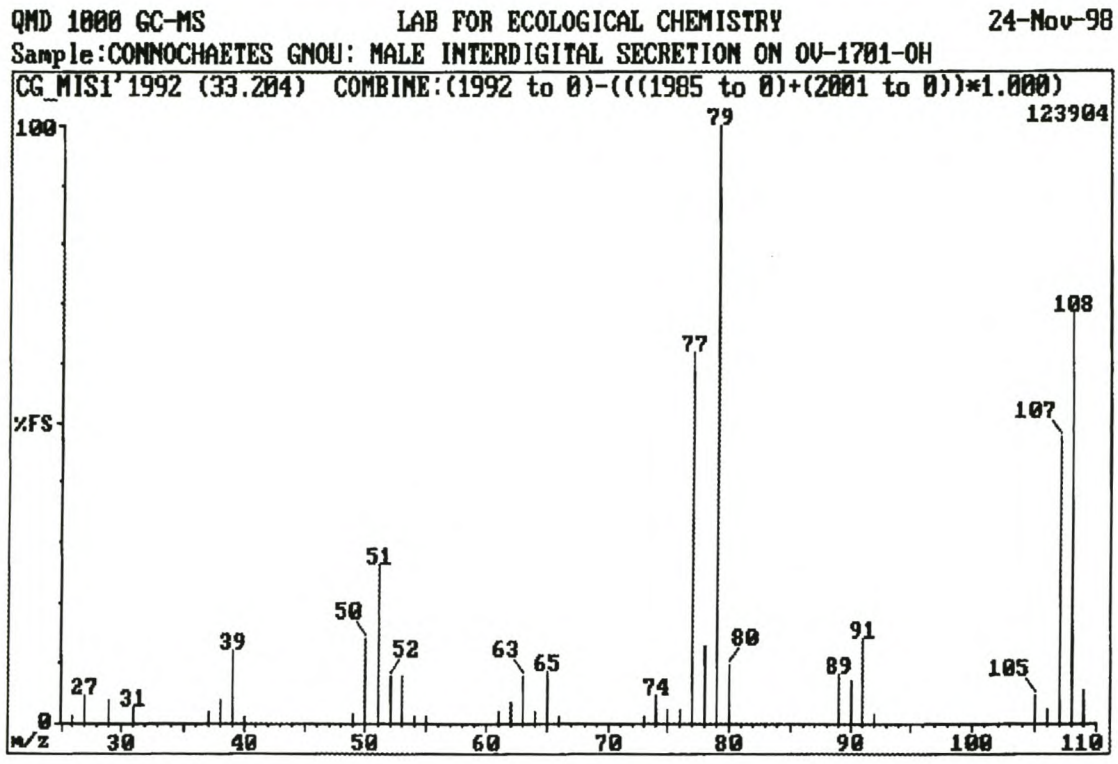


Fig. 2.25: EI mass spectrum of component 1992 (benzyl alcohol)

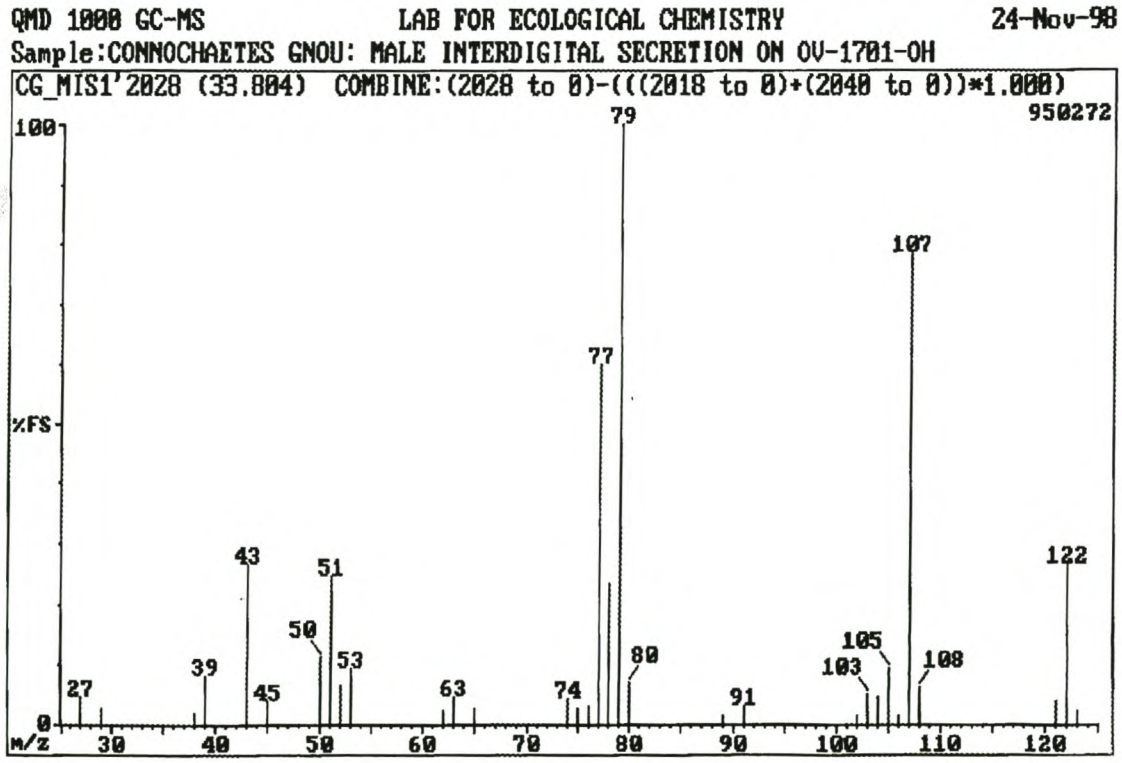


Fig. 2.26: EI mass spectrum of component 2028 (1-phenylethanol)

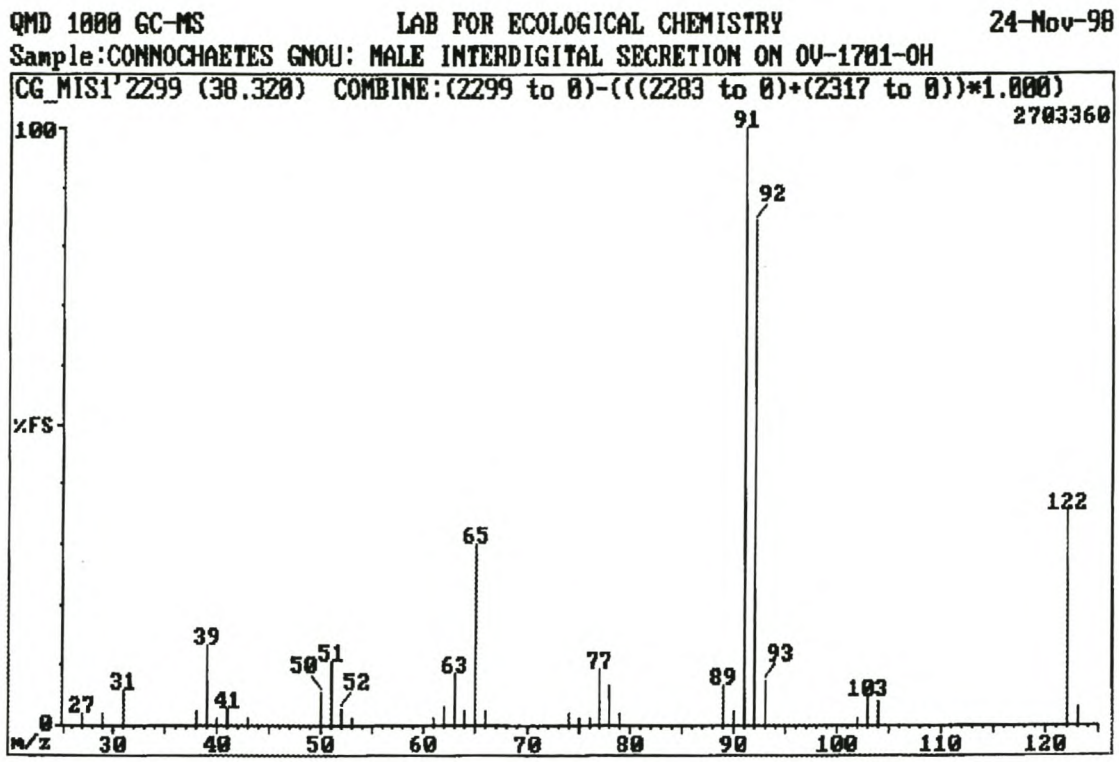


Fig. 2.27: EI mass spectrum of component 2299 (2-phenylethanol)

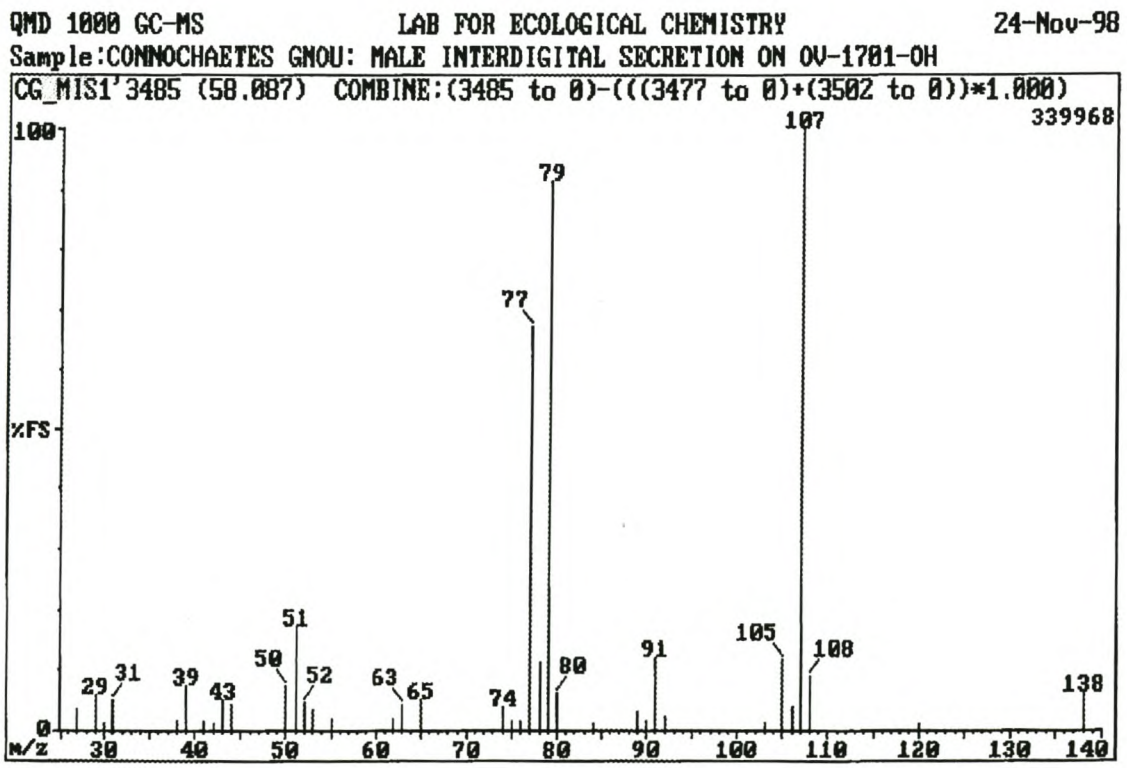


Fig. 2.28: EI mass spectrum of component 3485 (1-phenyl-1,2-ethanediol)

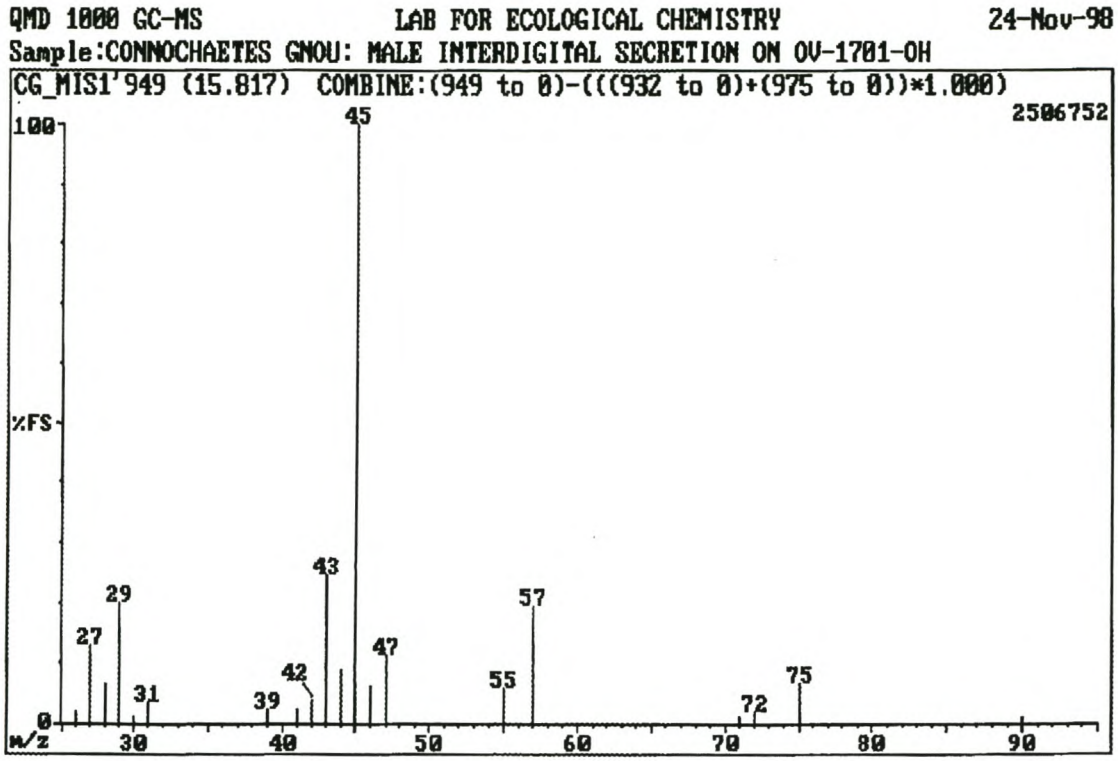


Fig. 2.29: EI mass spectrum of component 949 [(*R*,*R*)- and/or (*S*,*S*)-2,3-butanediol]

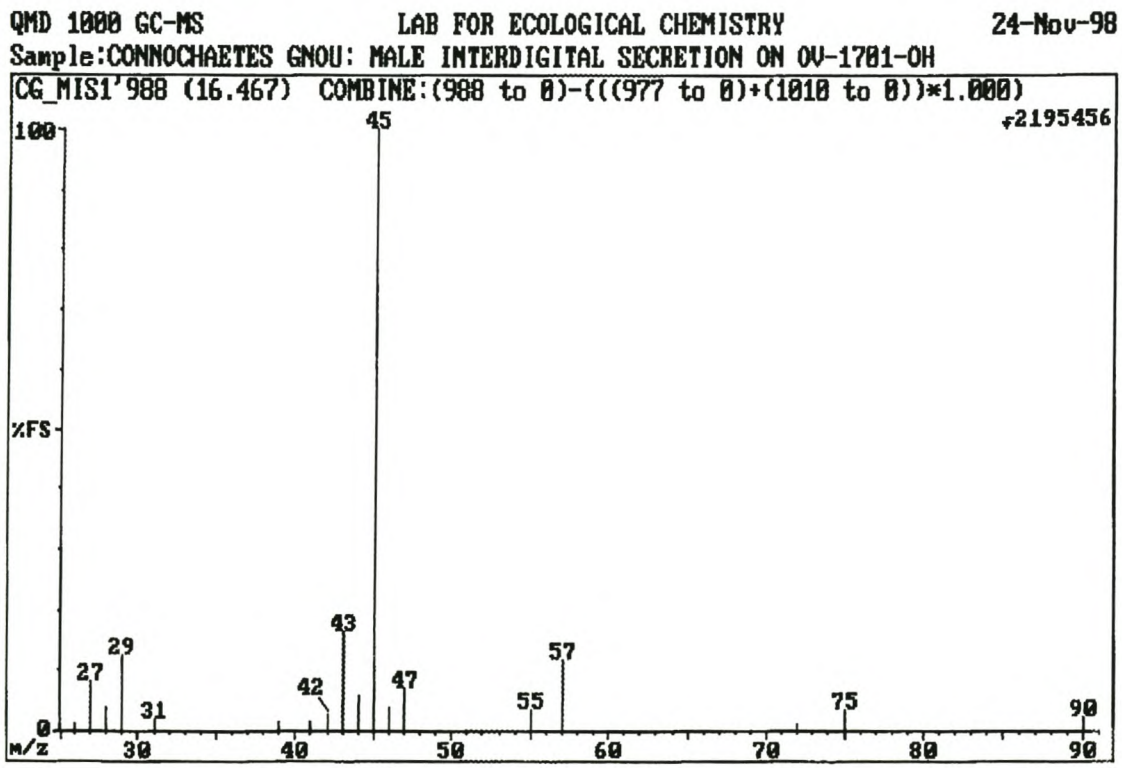


Fig. 2.30: EI mass spectrum of component 988 (*meso*-2,3-butanediol)

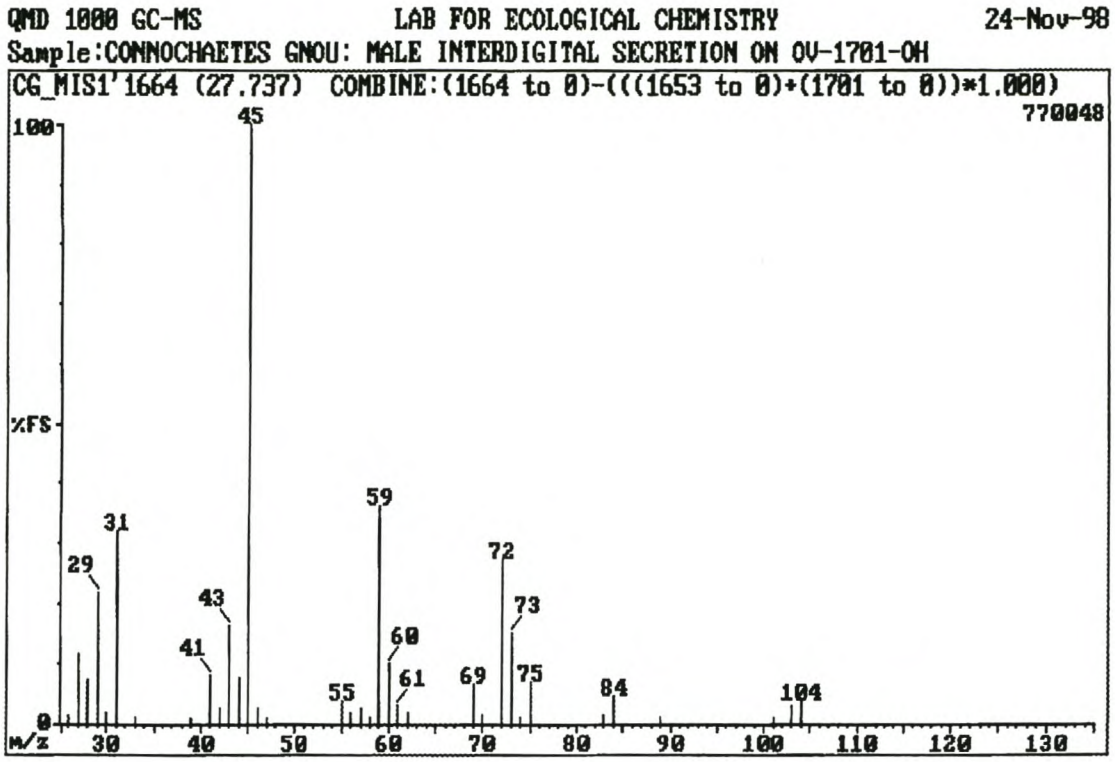


Fig. 2.31: EI mass spectrum of component 1664 [di(ethylene glycol)monoethyl ether]

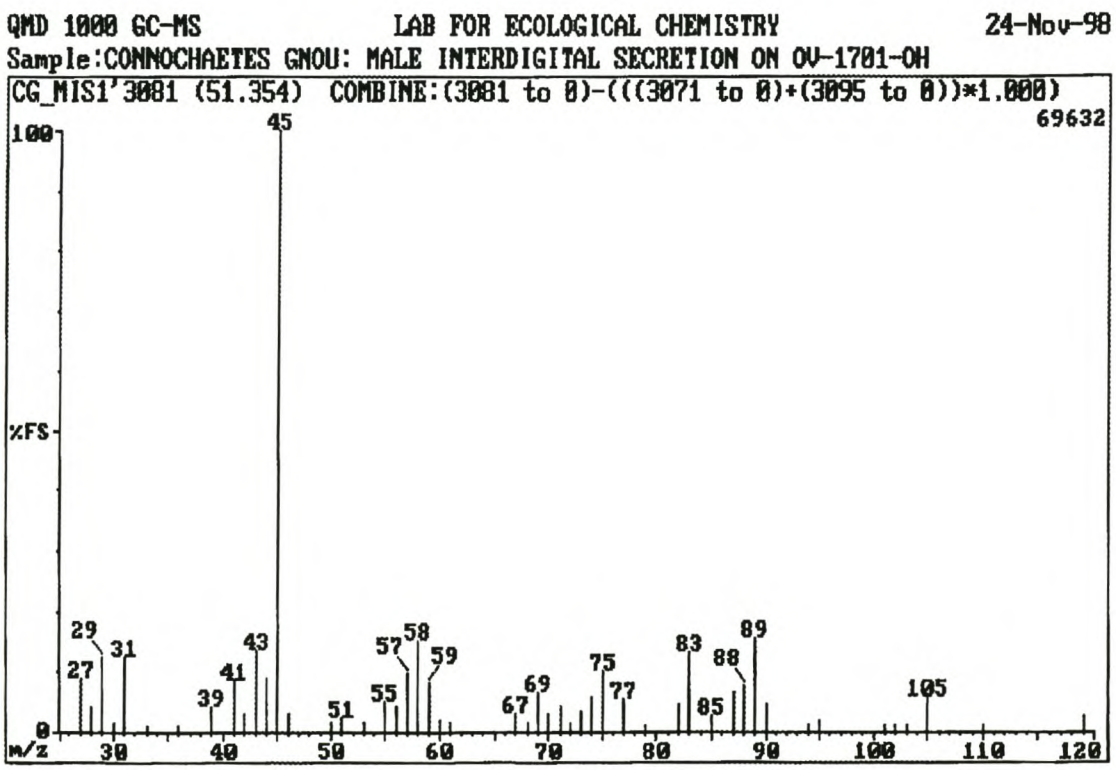


Fig. 2.32: EI mass spectrum of component 3081 [tri(ethylene glycol)]

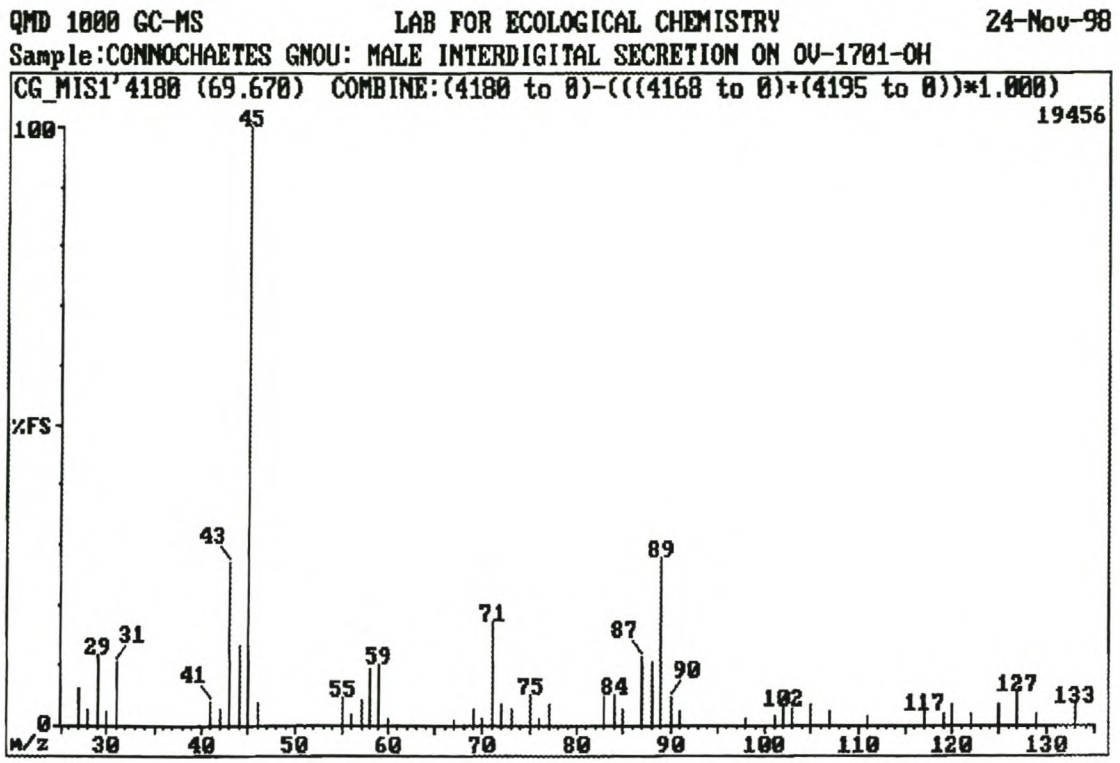


Fig. 2.33: EI mass spectrum of component 4180 [tetra(ethylene glycol)]

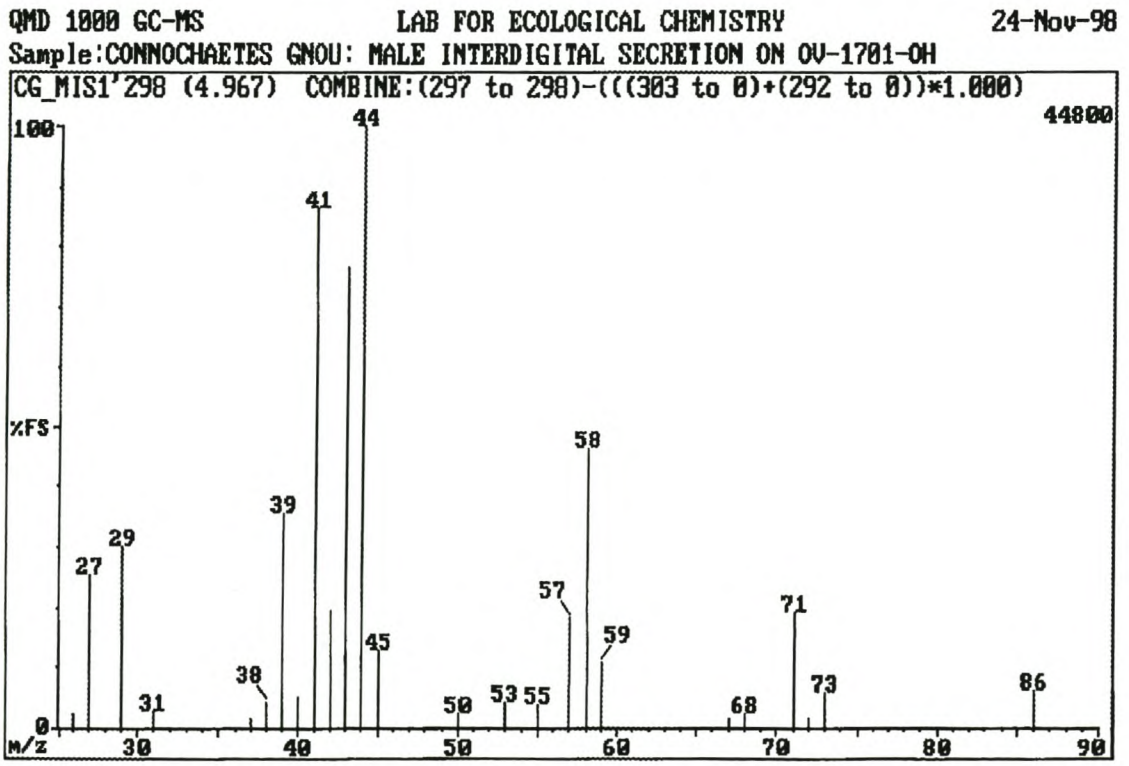


Fig. 2.34: EI mass spectrum of component 298 (3-methylbutanal)

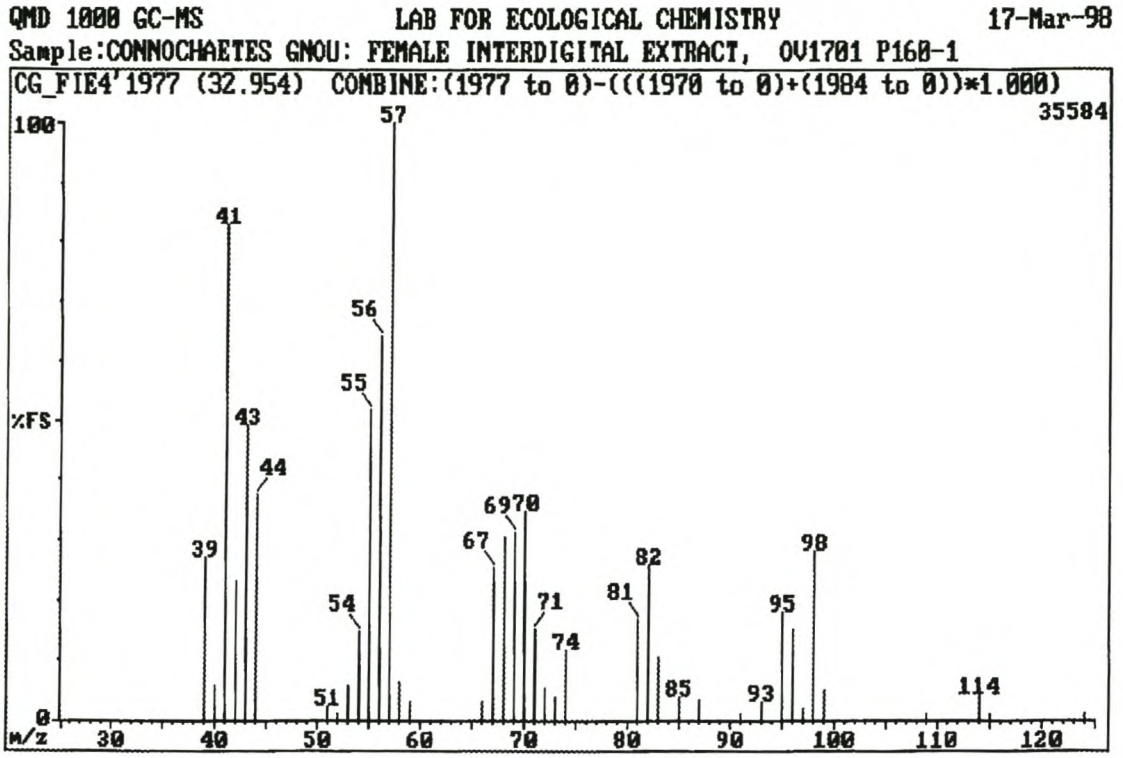


Fig. 2.35: EI mass spectrum of component 1909 (nonanal)

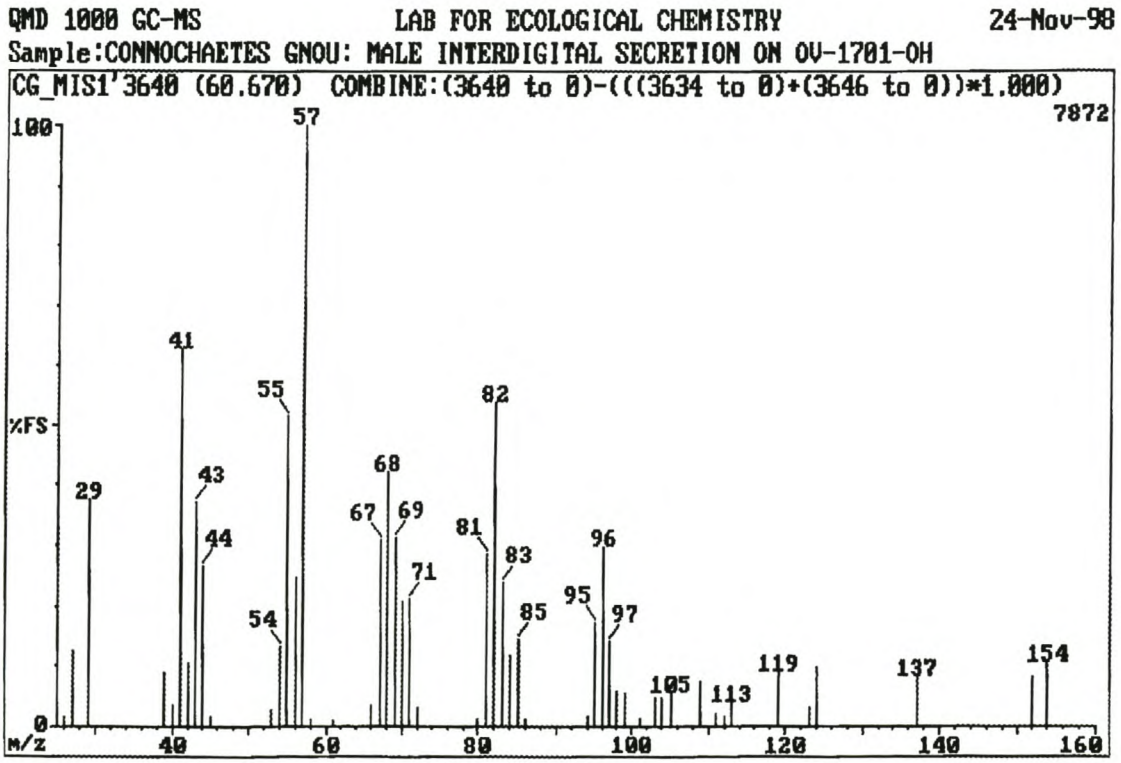


Fig. 2.36: EI mass spectrum of component 3640 (tridecanal)

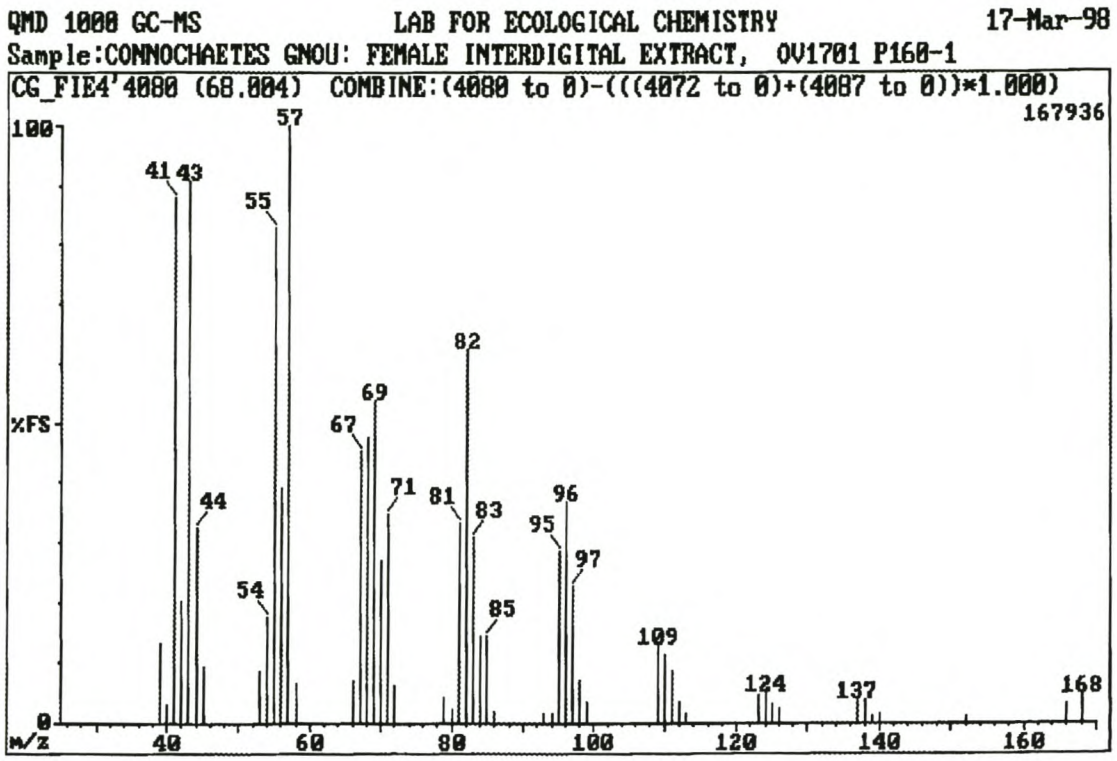


Fig. 2.37: EI mass spectrum of component 4021 (tetradecanal)

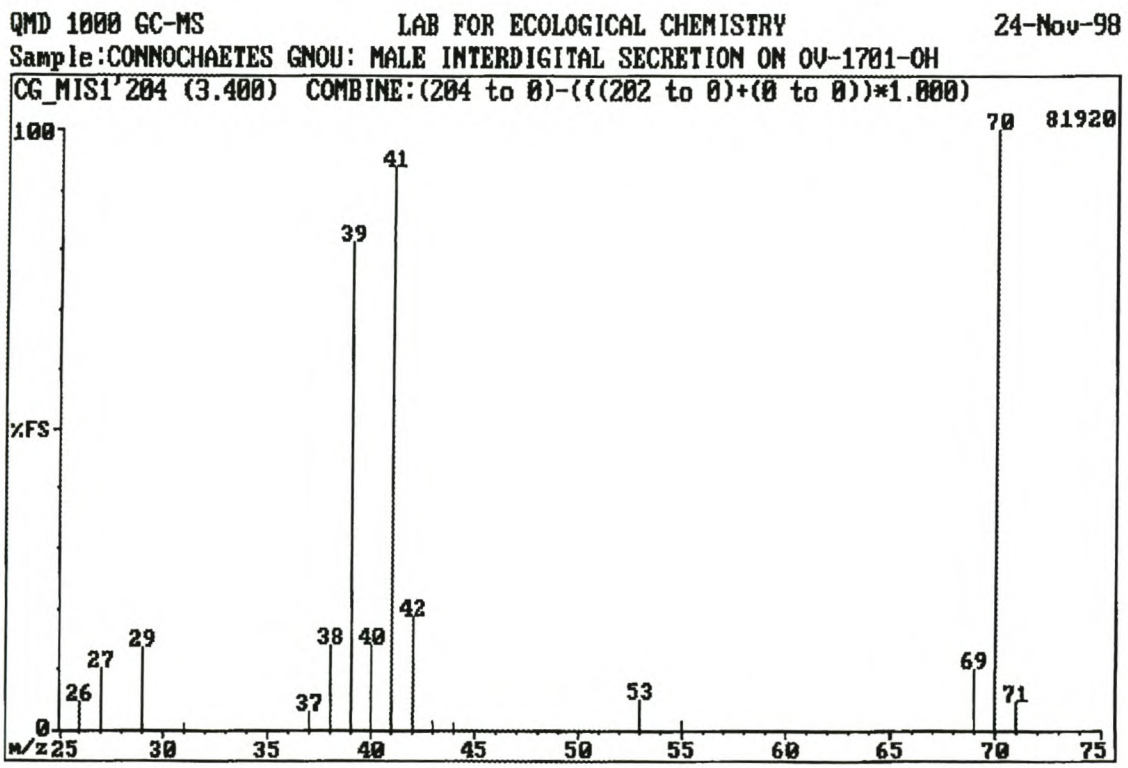


Fig. 2.38: EI mass spectrum of component 204 (2-methyl-2-propenal)

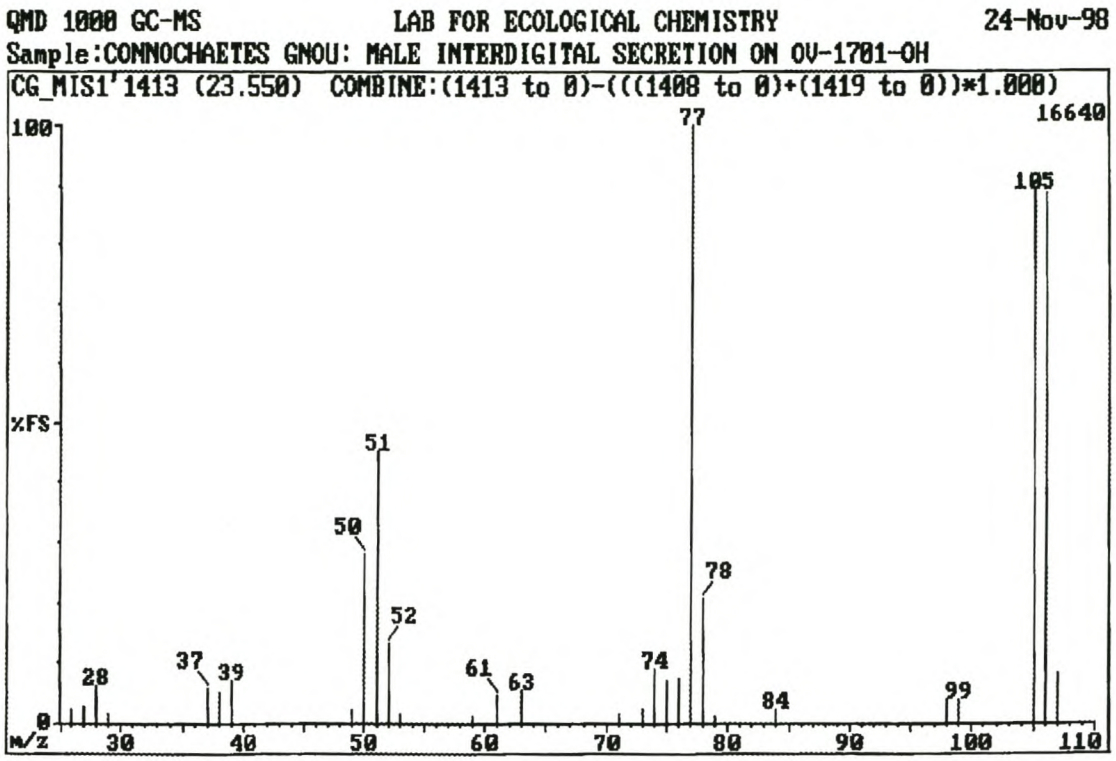


Fig. 2.39: EI mass spectrum of component 1413 (benzaldehyde)

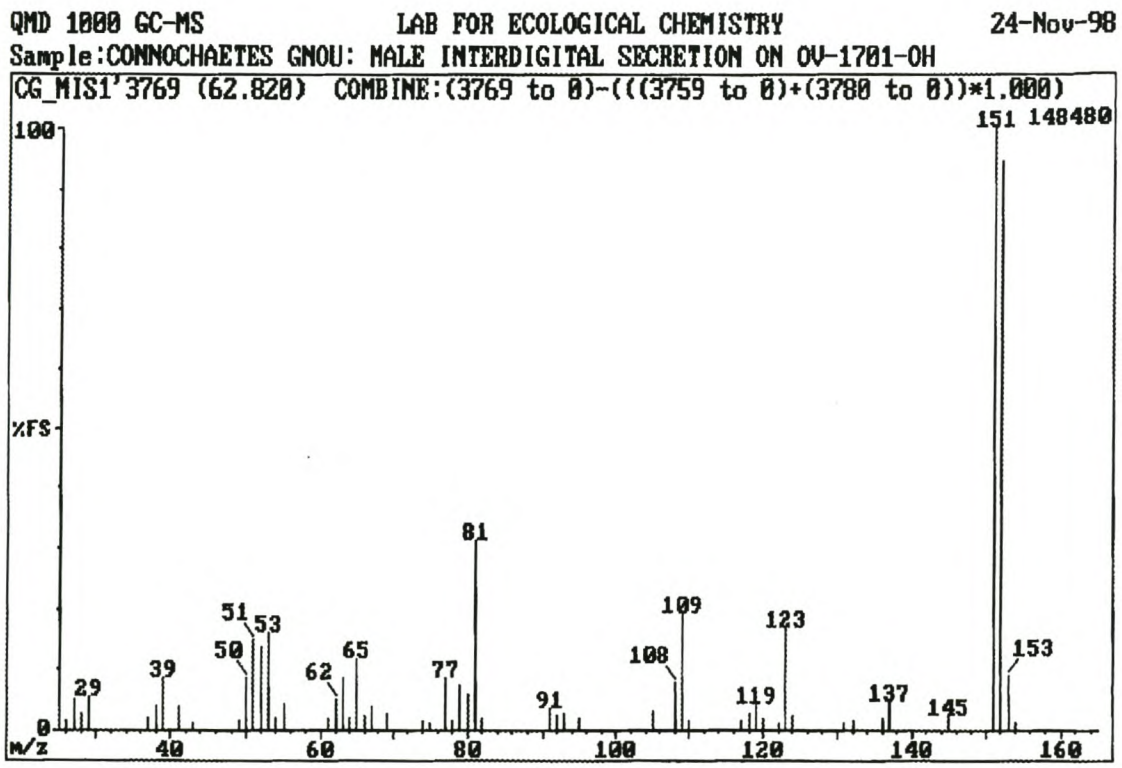


Fig. 2.40: EI mass spectrum of component 3769 (vanillin)

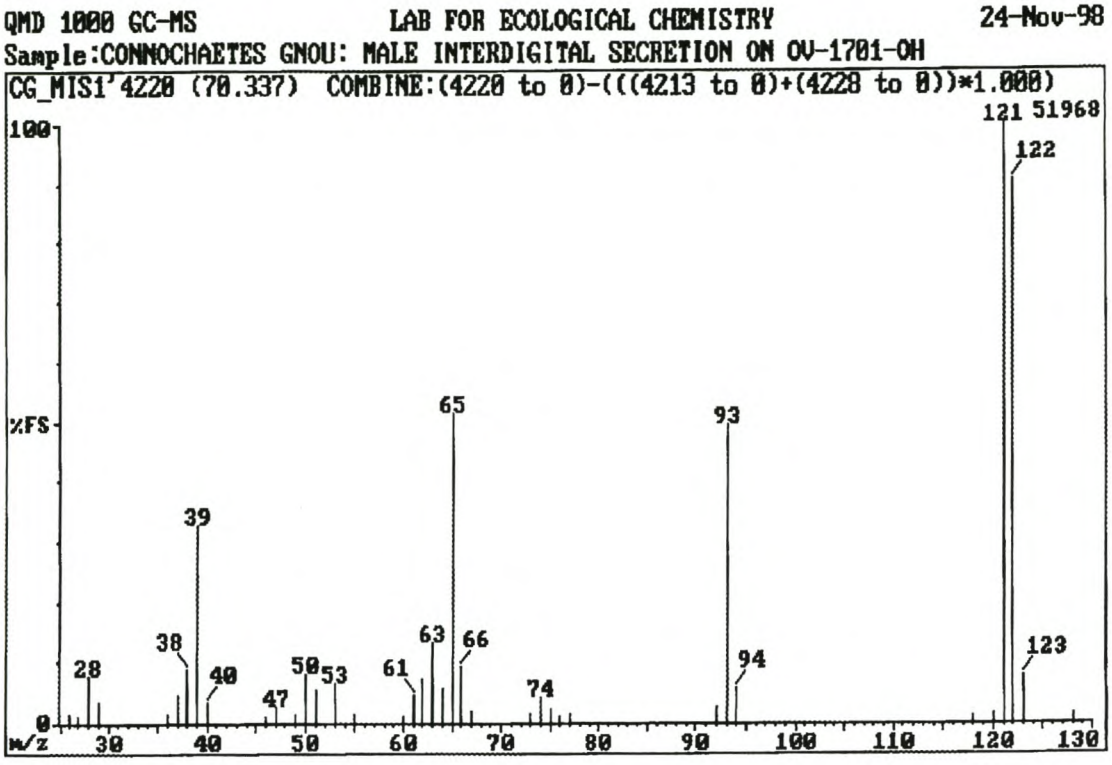


Fig. 2.41: EI mass spectrum of component 4220 (4-hydroxybenzaldehyde)

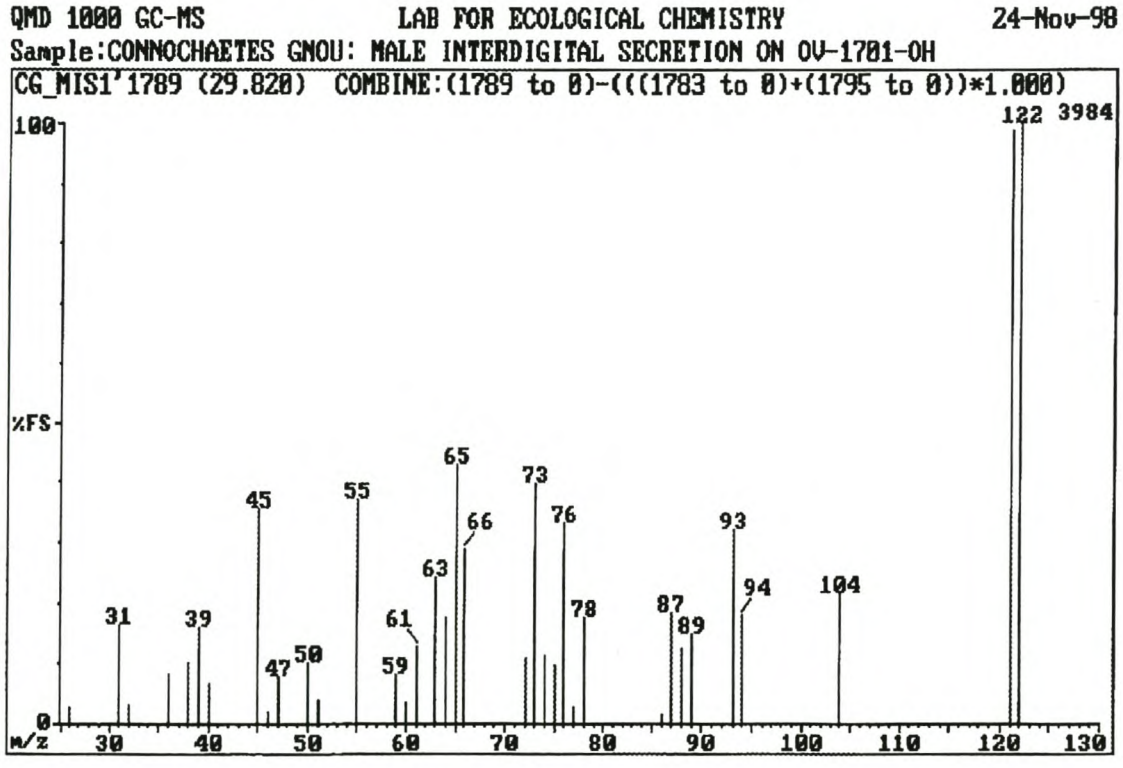


Fig. 2.42: EI mass spectrum of component 1789 (2-hydroxybenzaldehyde)

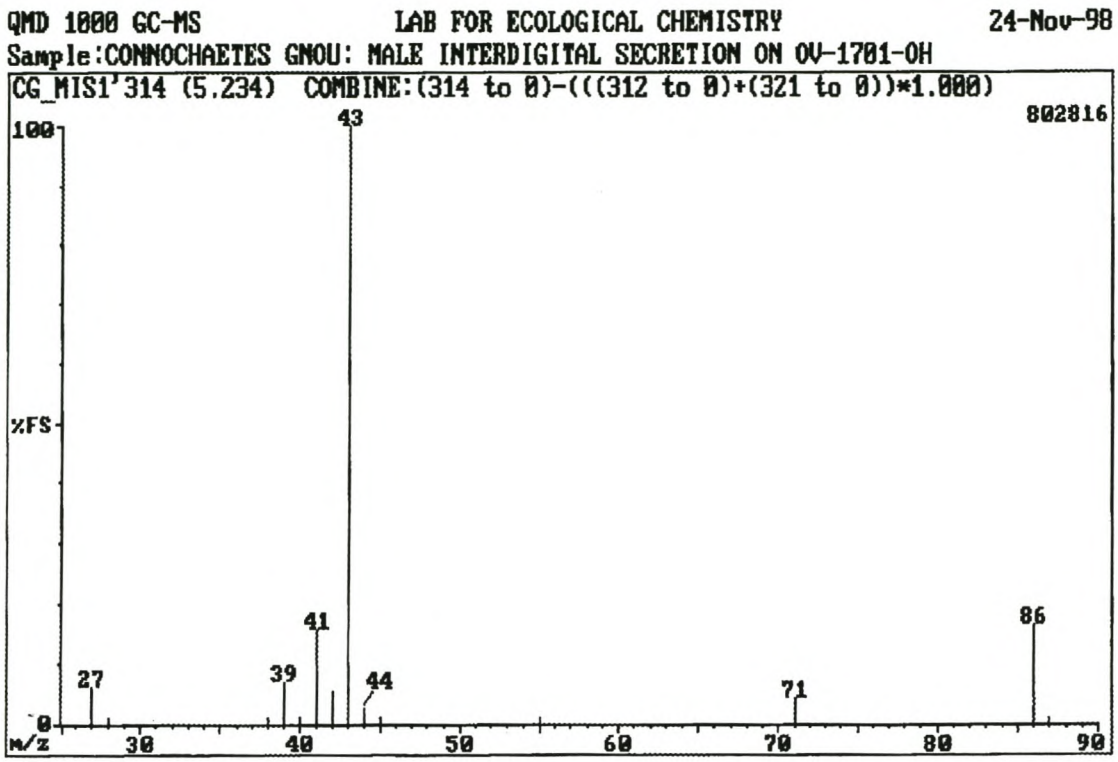


Fig. 2.43: EI mass spectrum of component 314 (3-methyl-2-butanone)

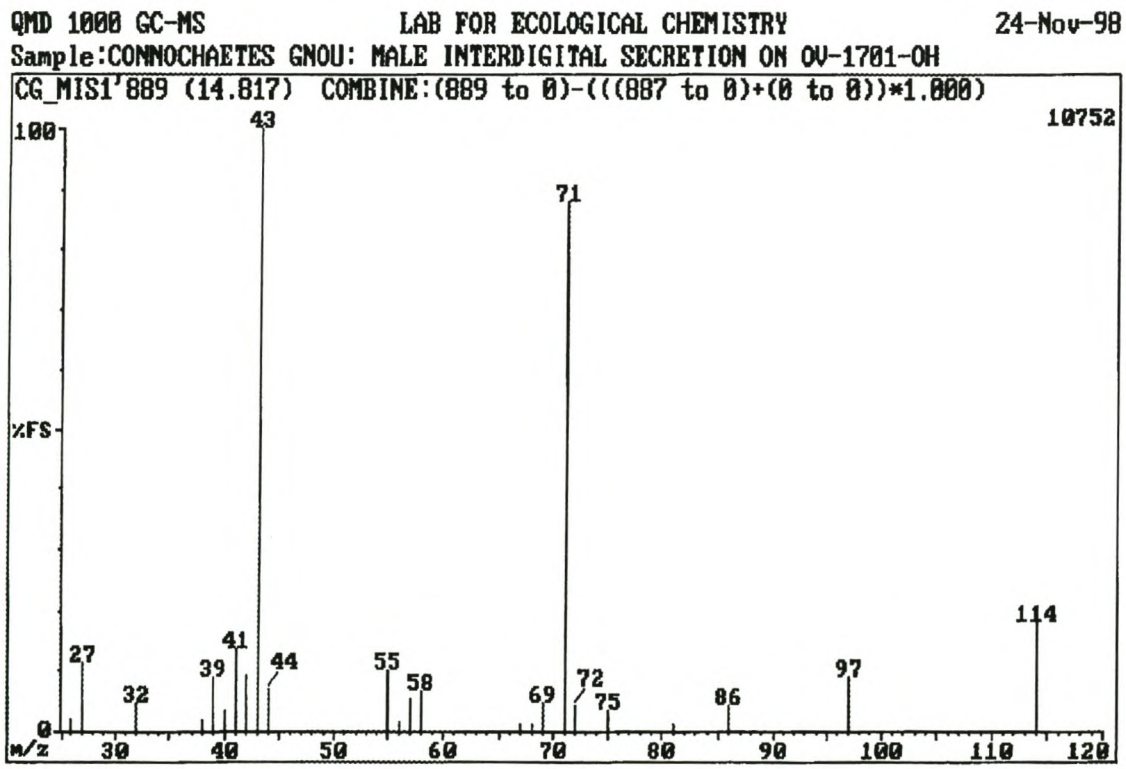


Fig. 2.44: EI mass spectrum of component 889 (4-heptanone)

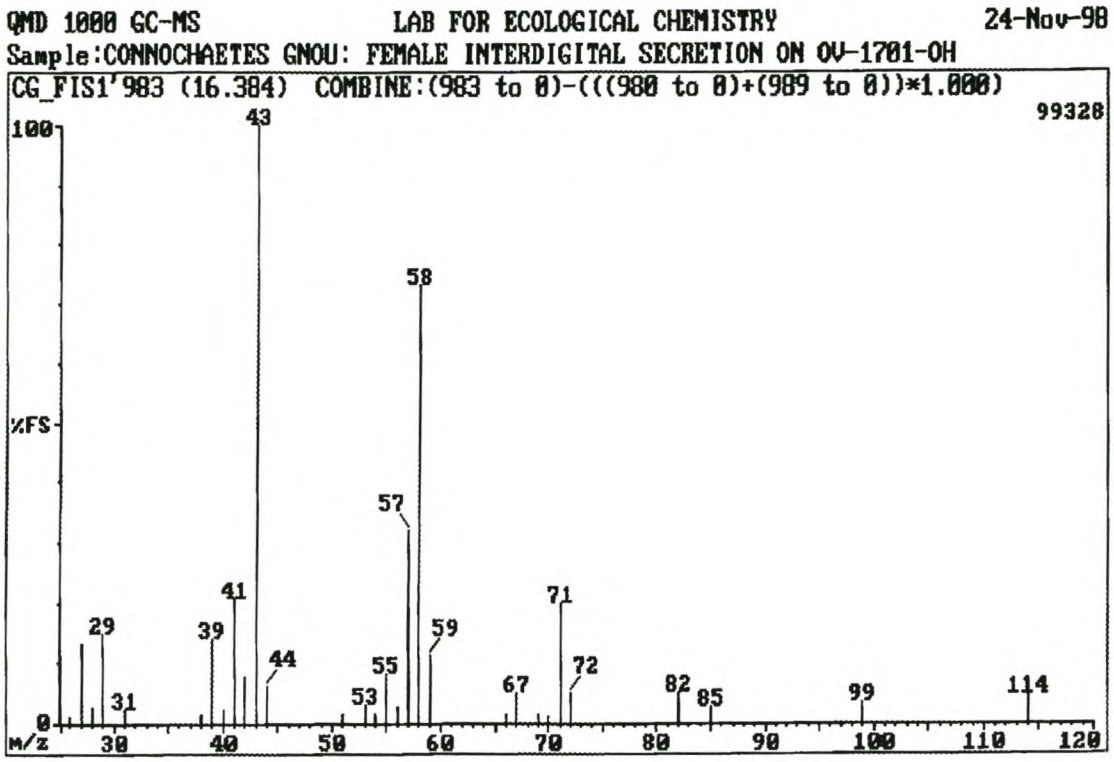


Fig. 2.45: EI mass spectrum of component 983 (2-heptanone)

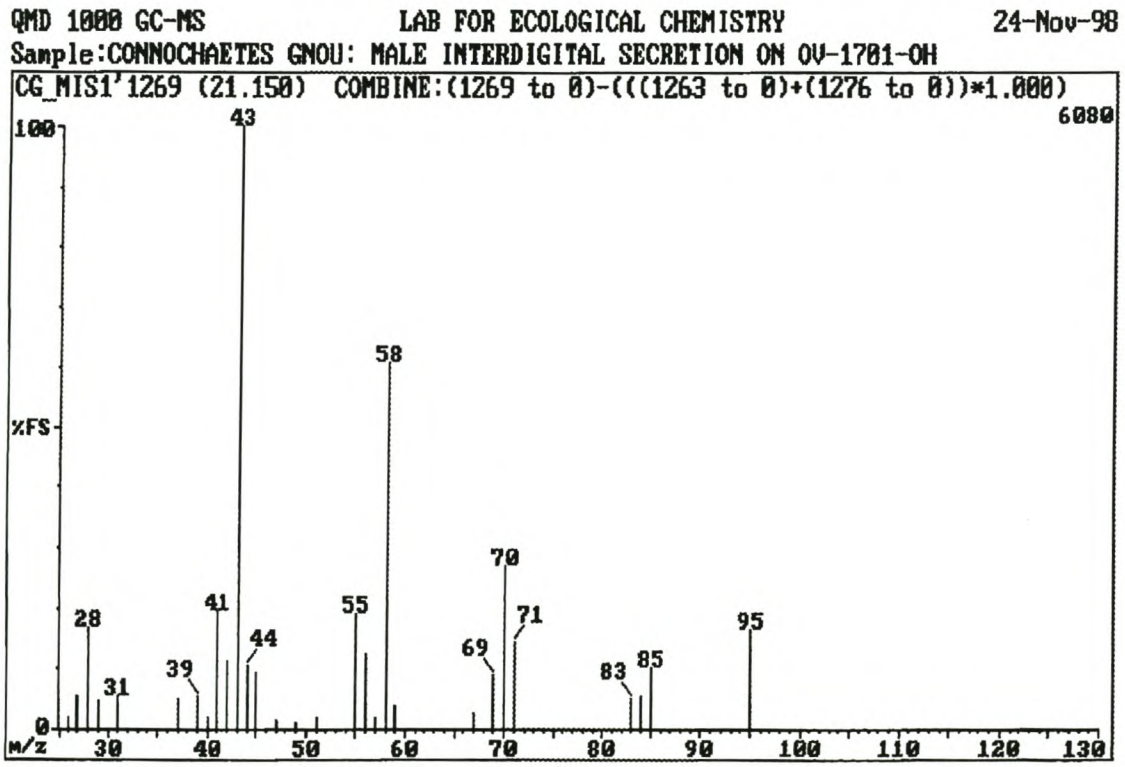


Fig. 2.46: EI mass spectrum of component 1269 (6-methyl-2-heptanone)

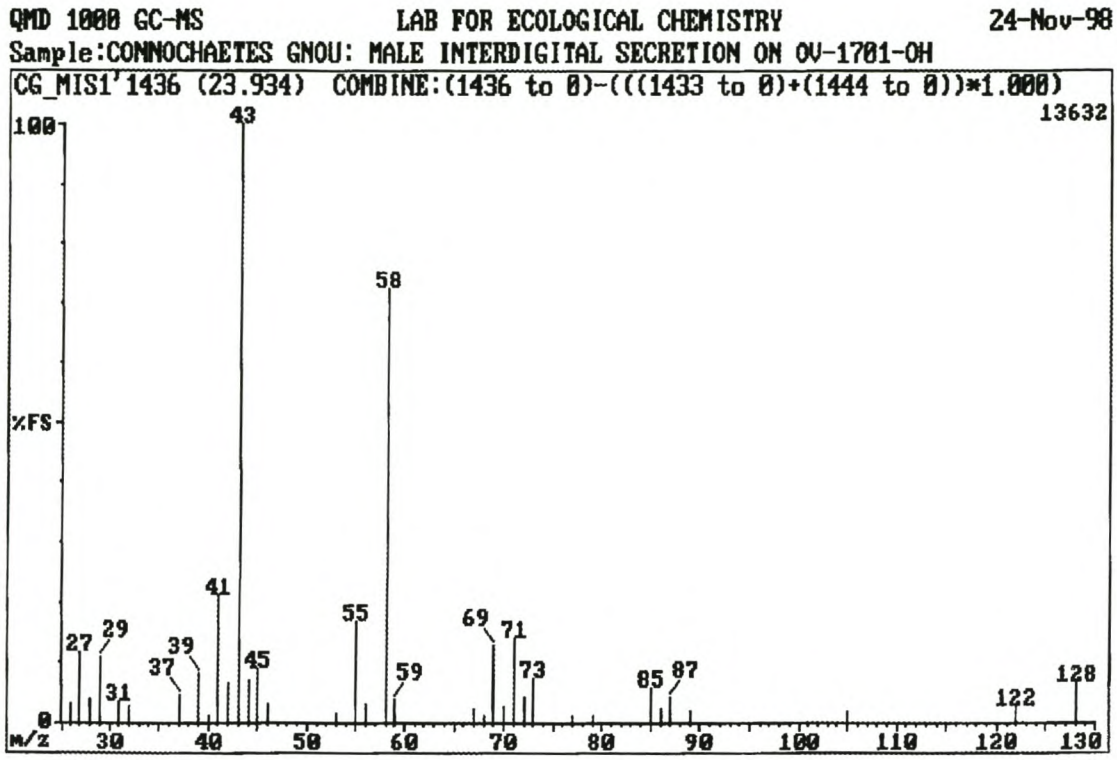


Fig. 2.47: EI mass spectrum of component 1436 (2-octanone)

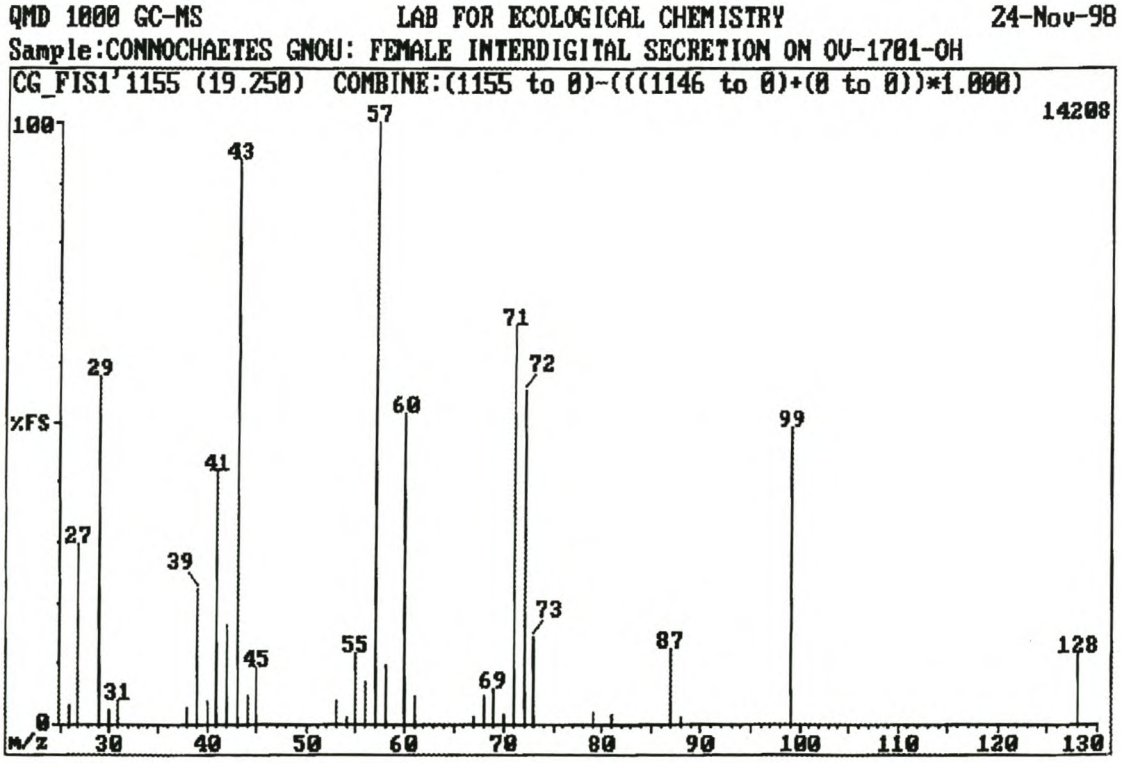


Fig. 2.48: EI mass spectrum of component 1169 (3-octanone)

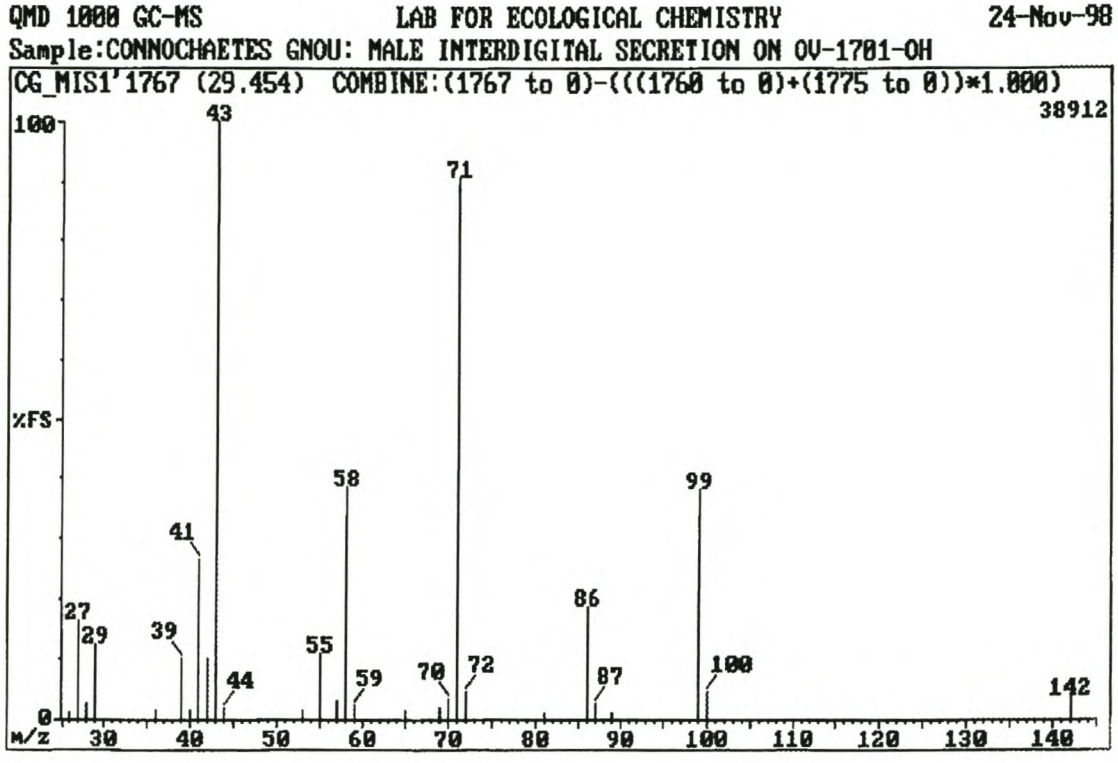


Fig. 2.49: EI mass spectrum of component 1766 (4-nonanone)

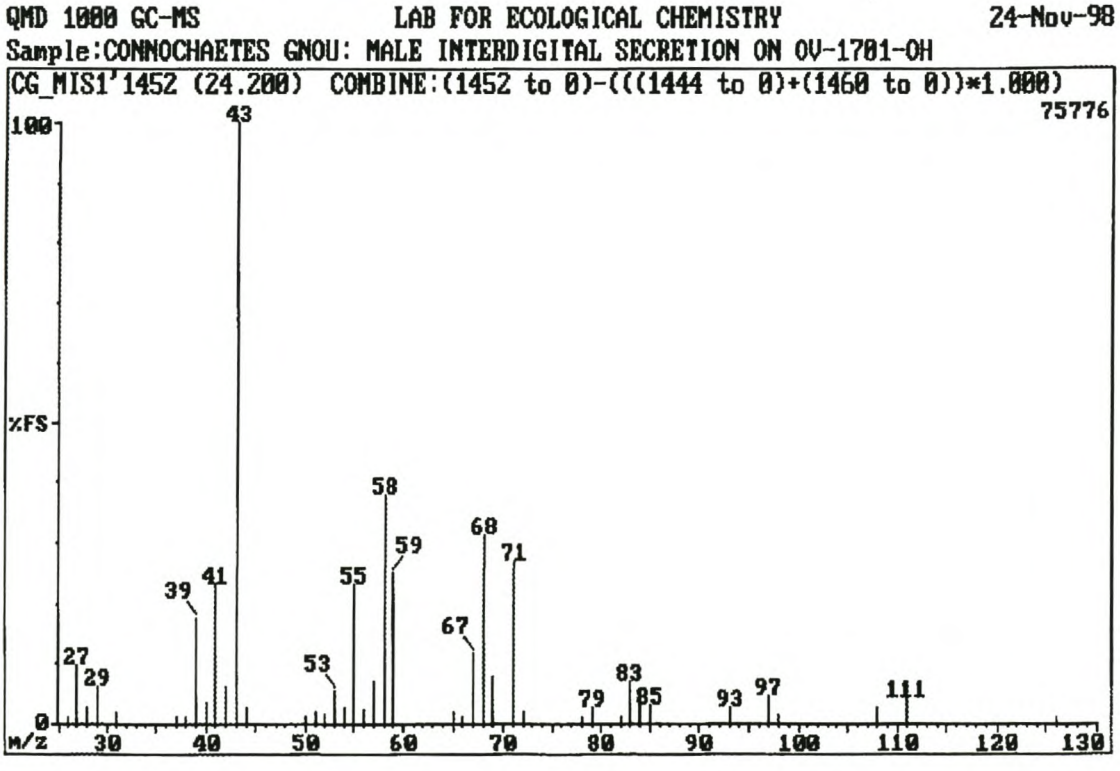


Fig. 2.50: EI mass spectrum of component 1452 (7-octen-2-one)

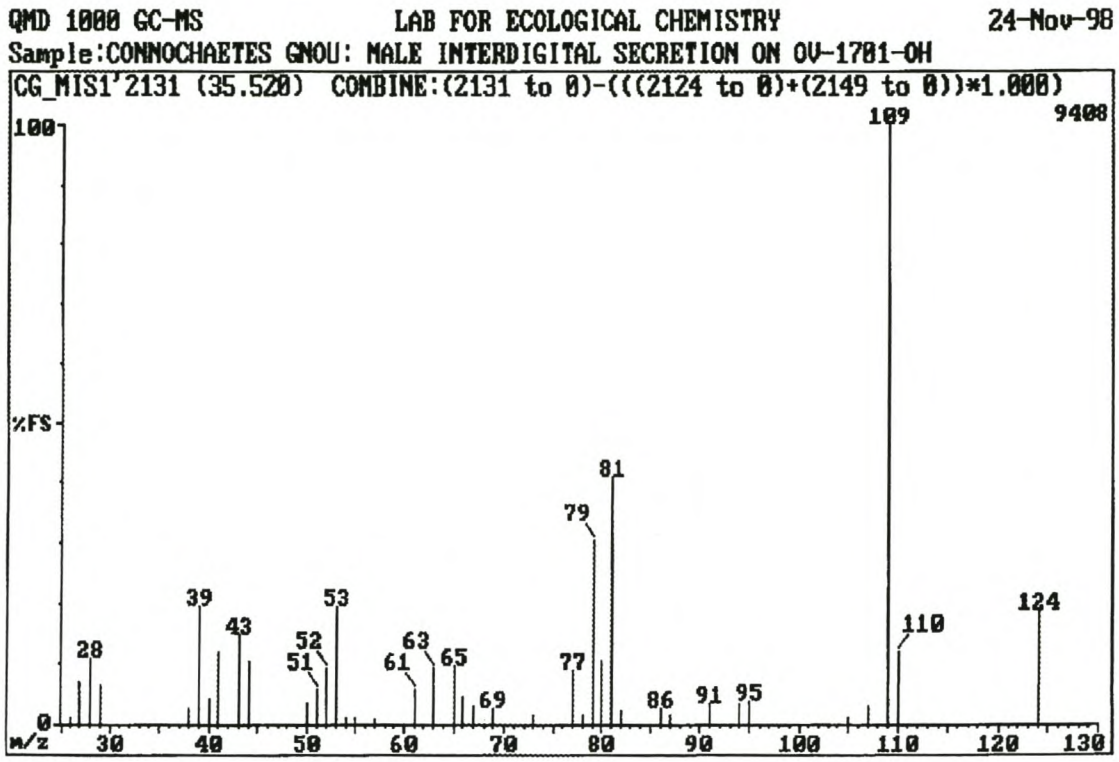


Fig. 2.51: EI mass spectrum of component 2131 (*trans*-6-methyl-3,5-heptadien-2-one)

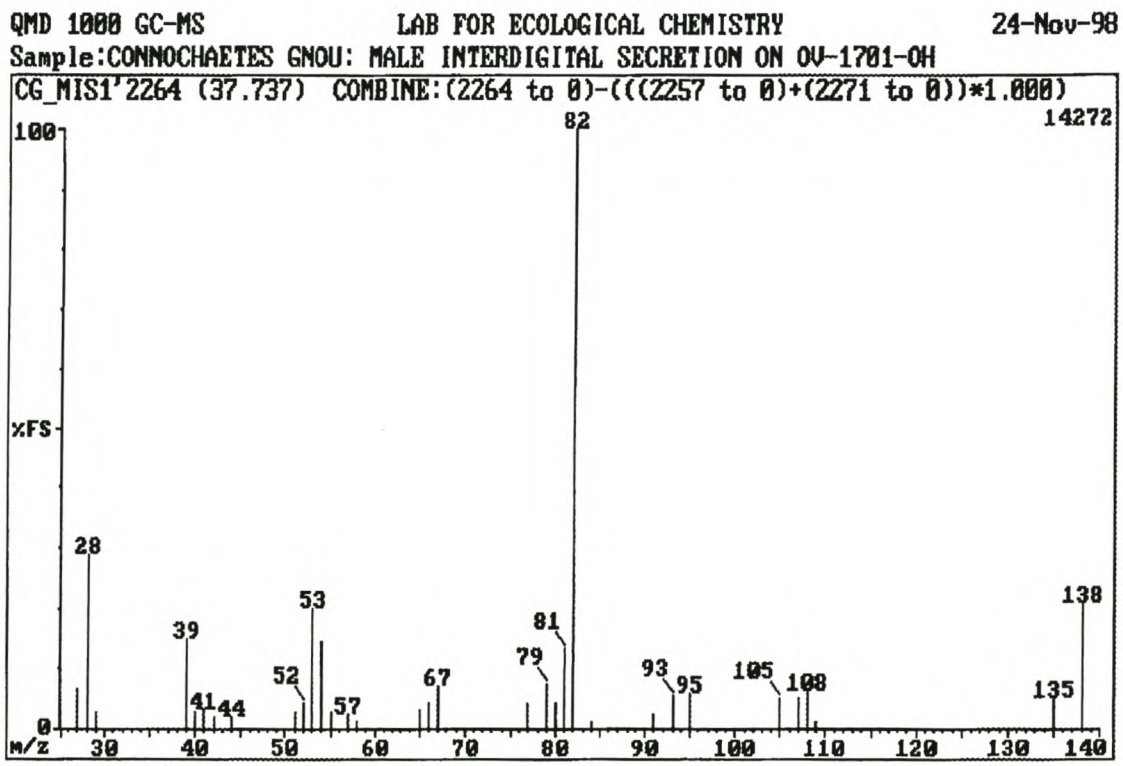


Fig. 2.52: EI mass spectrum of component 2264 (isophorone)

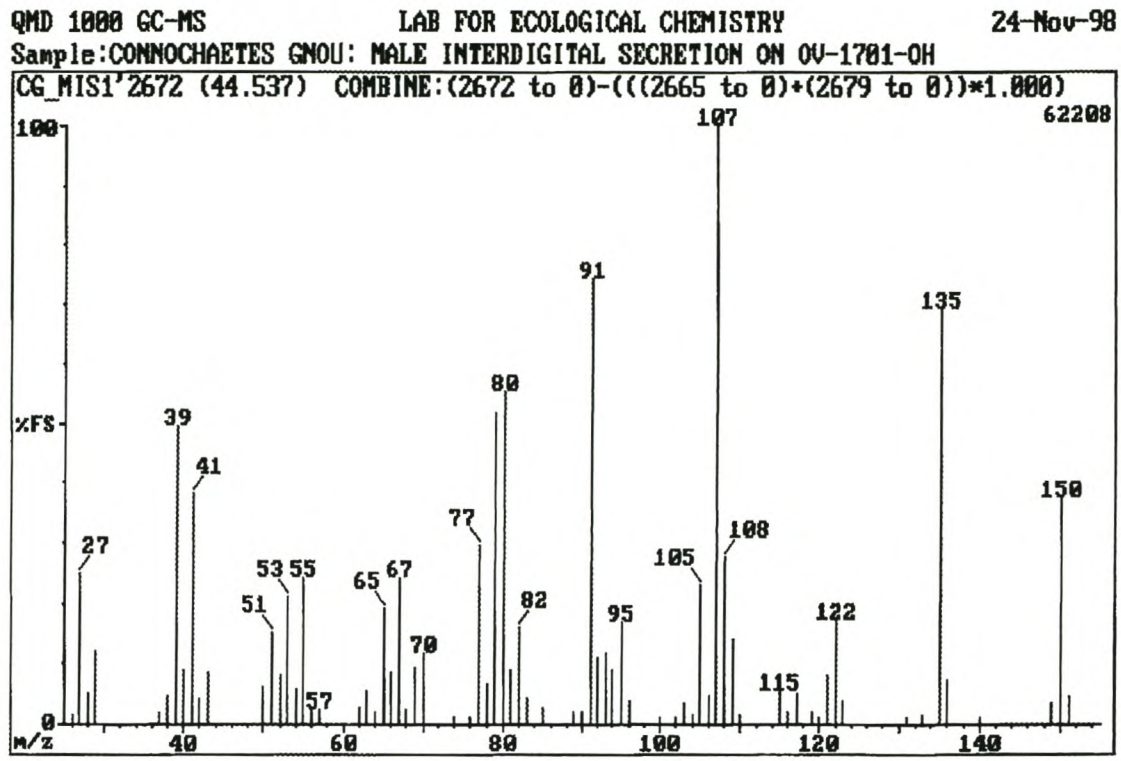


Fig. 2.53: EI mass spectrum of component 2672 (verbenone)

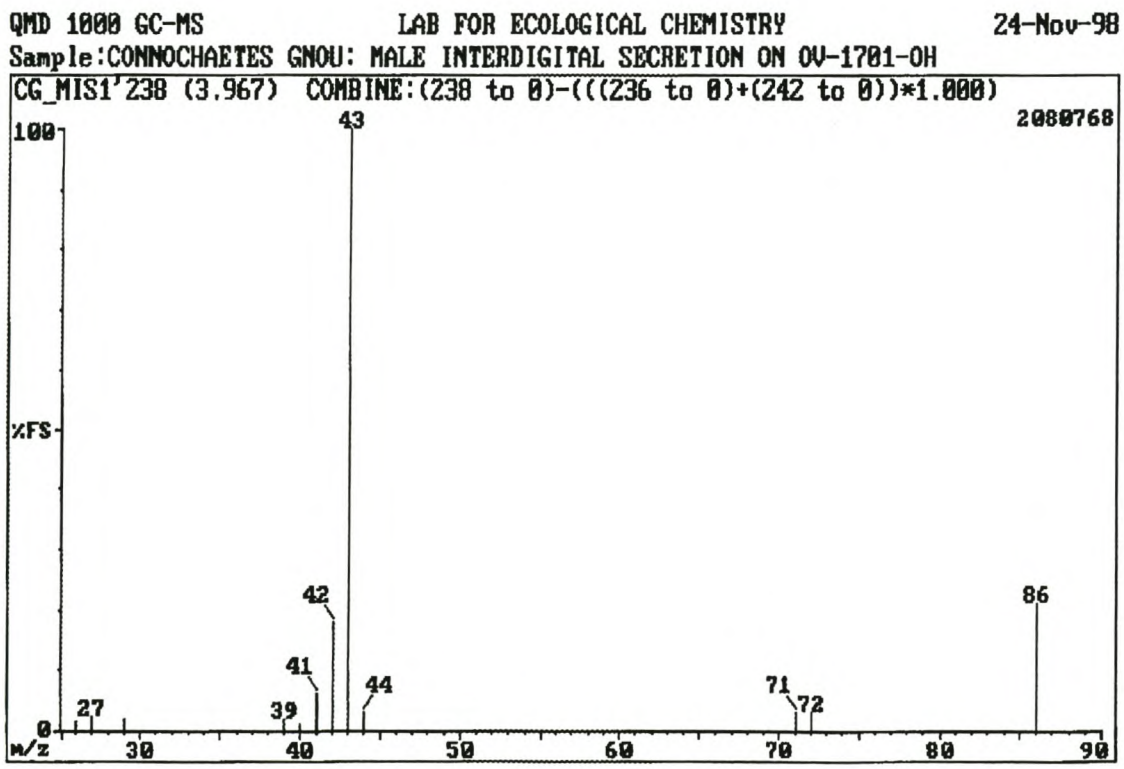


Fig. 2.54: EI mass spectrum of component 238 (2,3-butanedione)

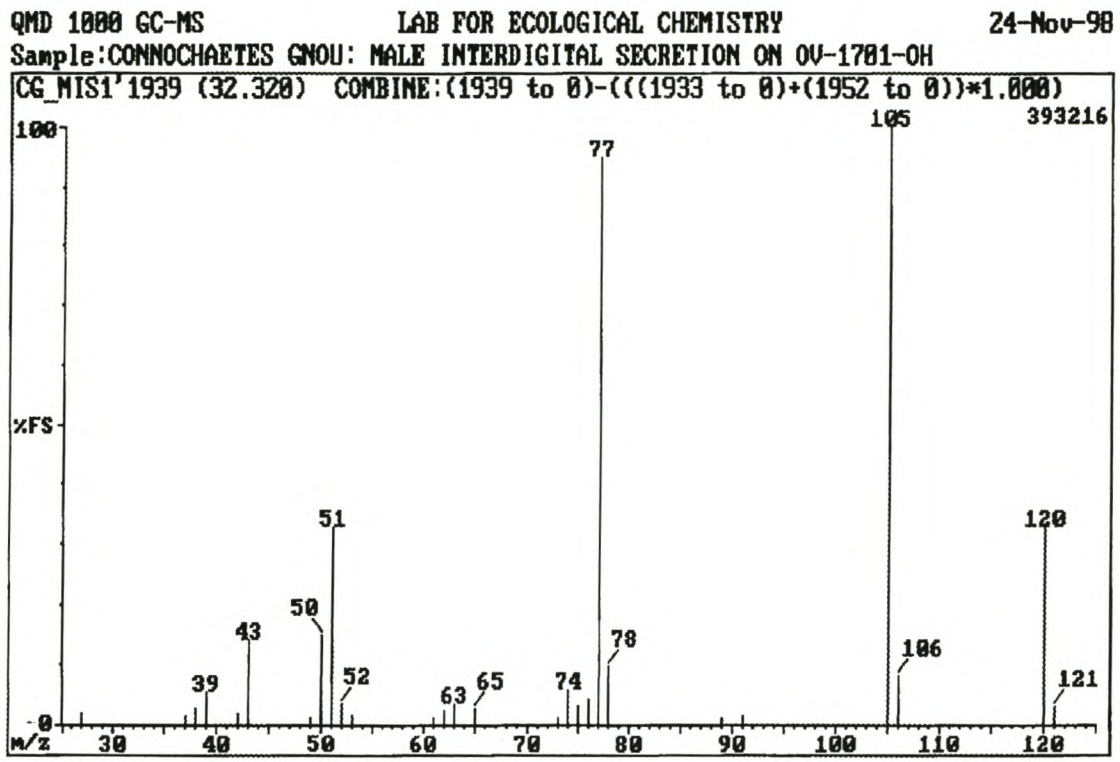


Fig. 2.55: EI mass spectrum of component 1939 (acetophenone)

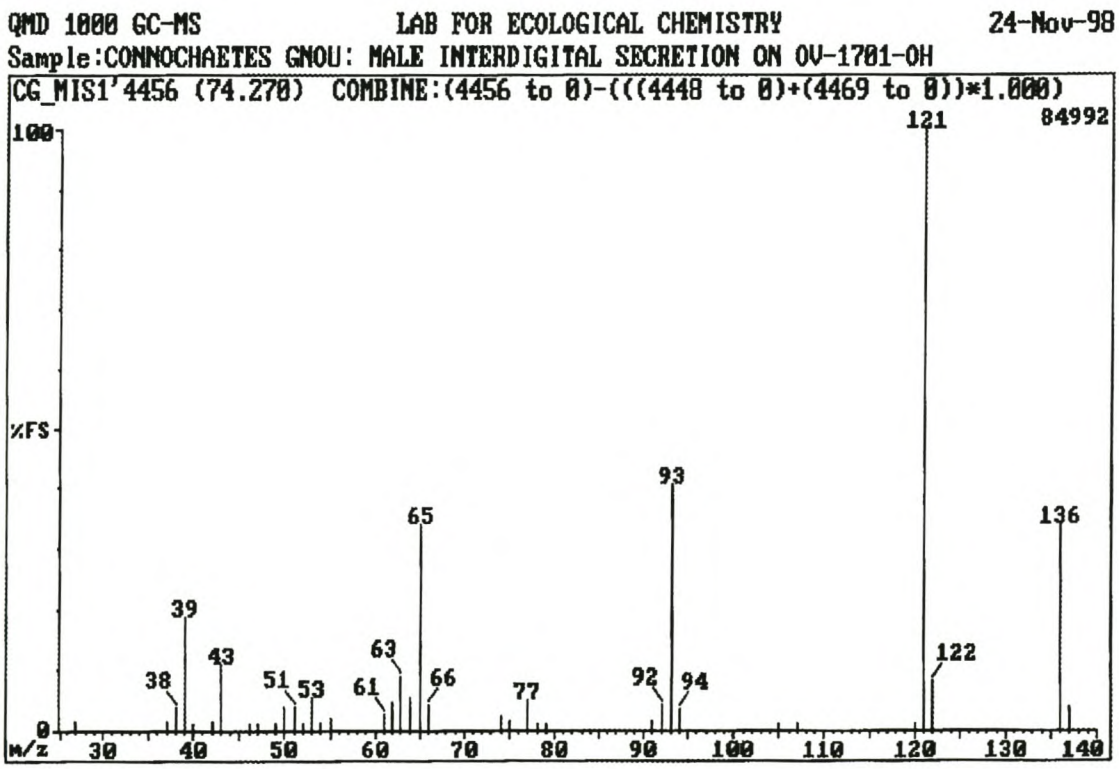


Fig. 2.56: EI mass spectrum of component 4456 (4-hydroxyacetophenone)

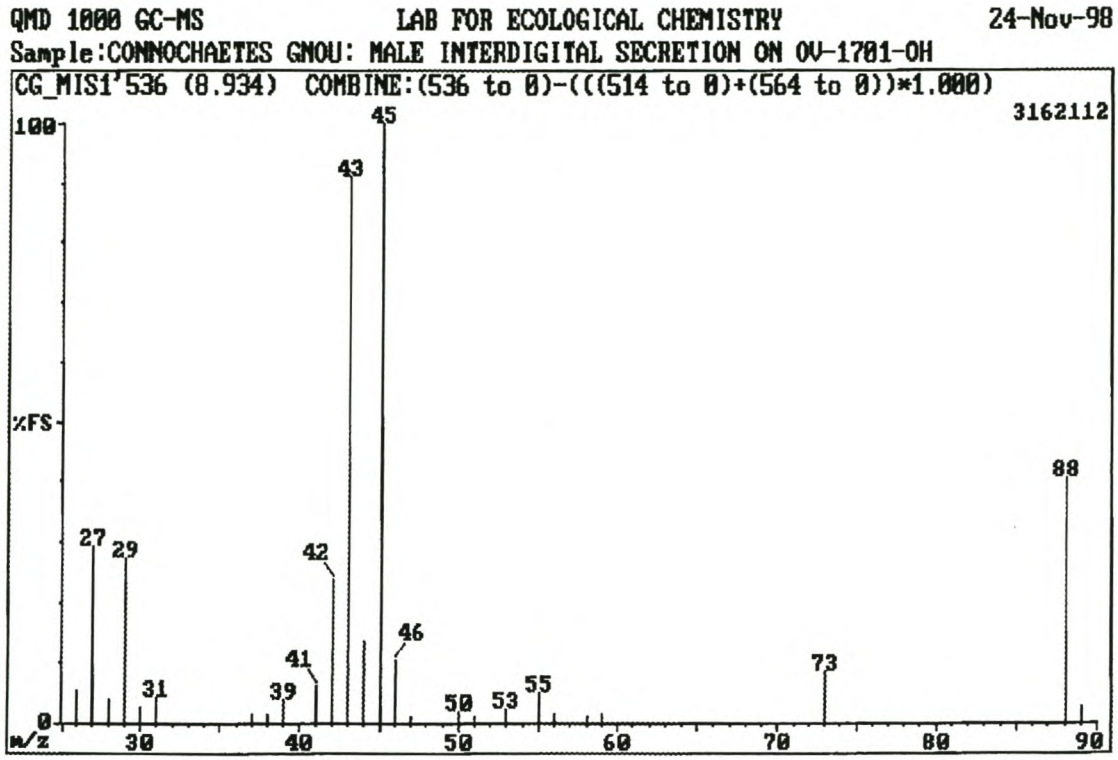


Fig. 2.57: EI mass spectrum of component 536 (3-hydroxy-2-butanone)

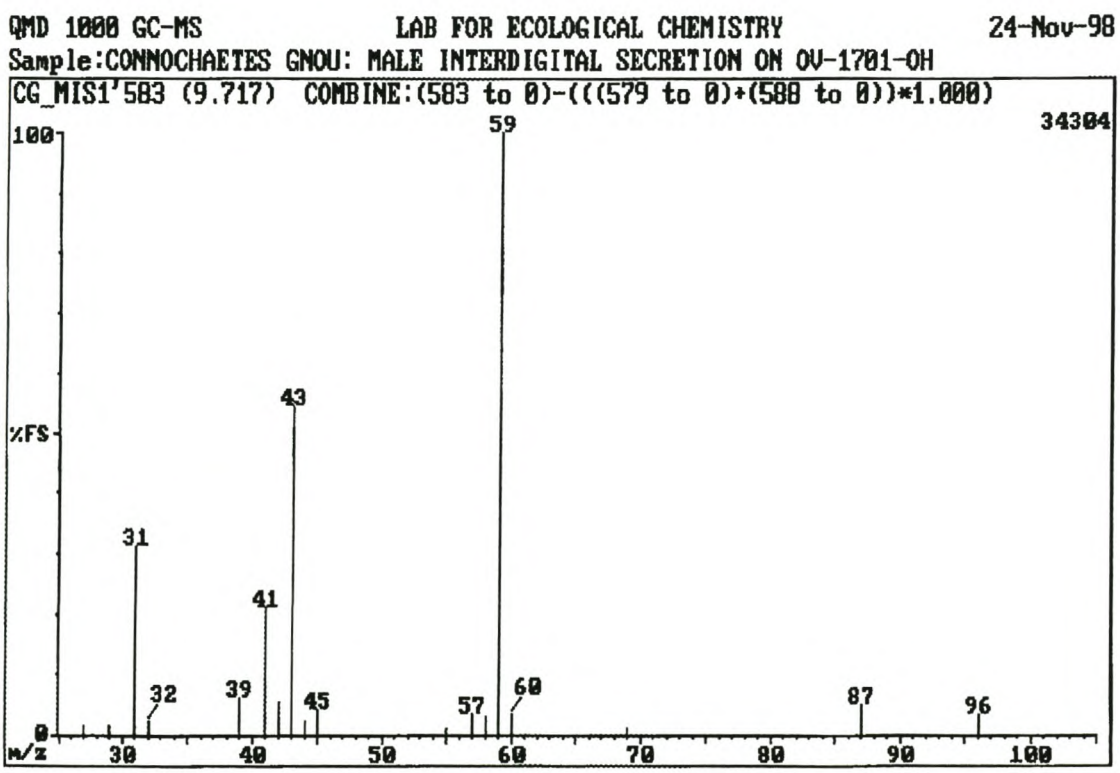


Fig. 2.58: EI mass spectrum of component 583 (3-hydroxy-3-methyl-2-butanone)

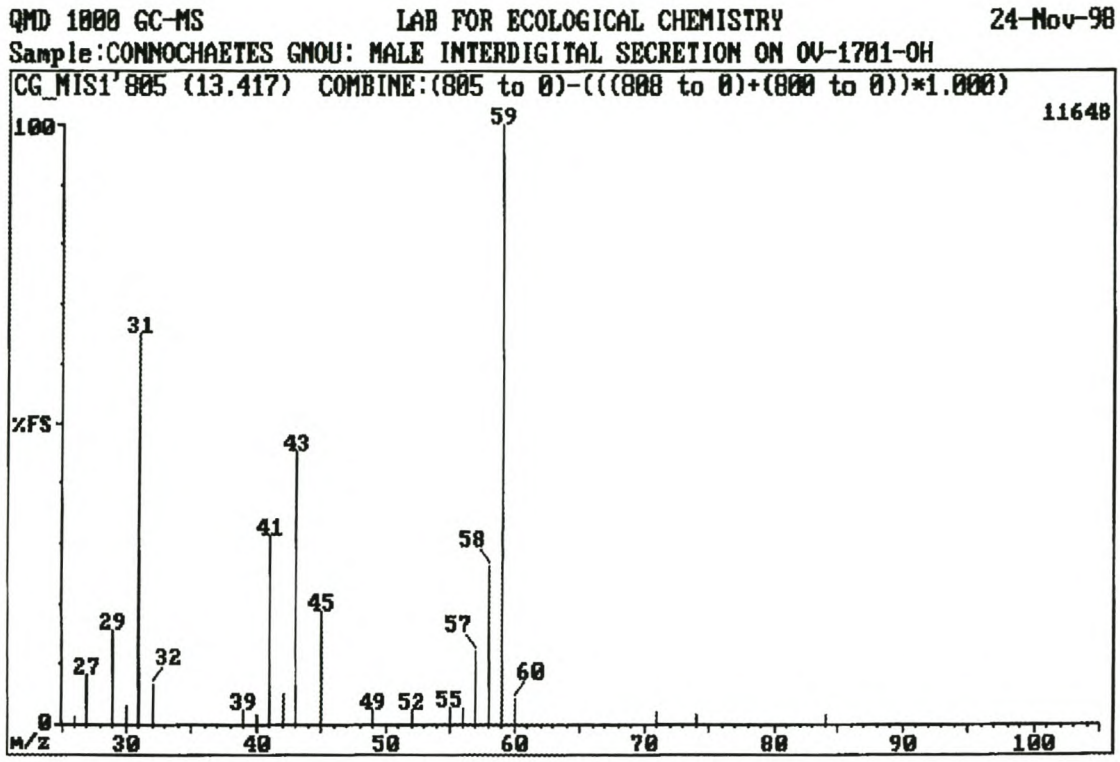


Fig. 2.59: EI mass spectrum of component 805 (3-hydroxy-2-pentanone)

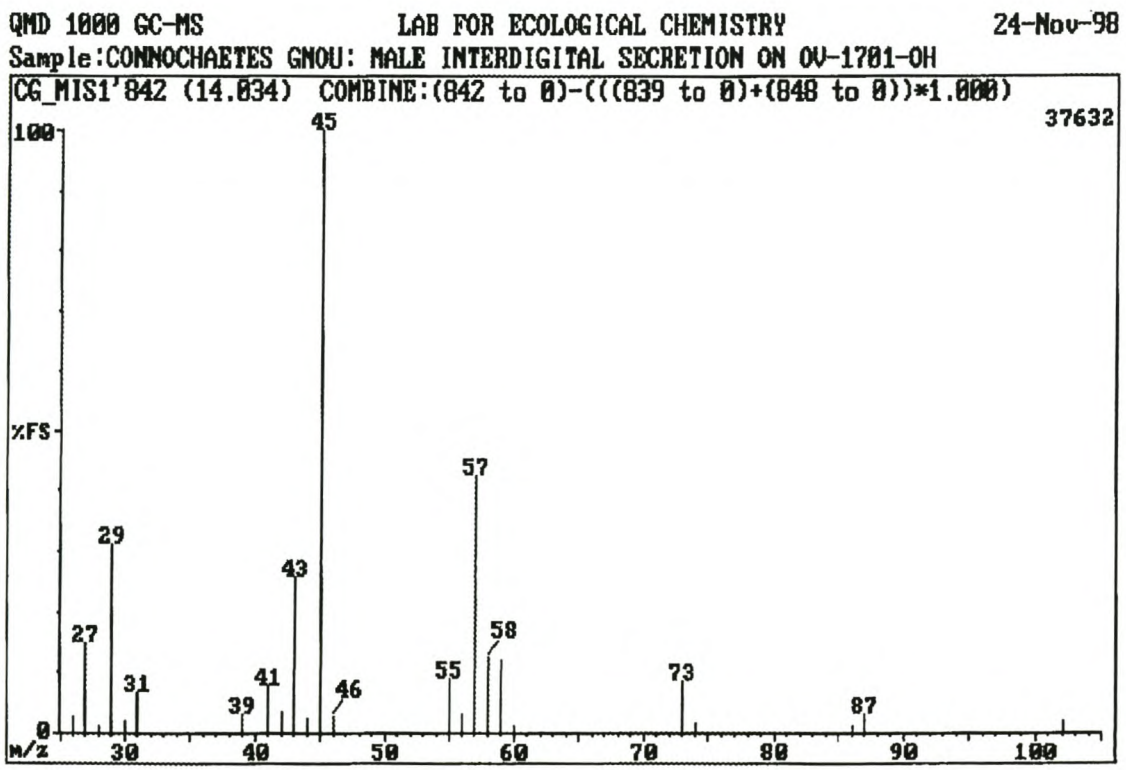


Fig. 2.60: EI mass spectrum of component 842 (2-hydroxy-3-pentanone)

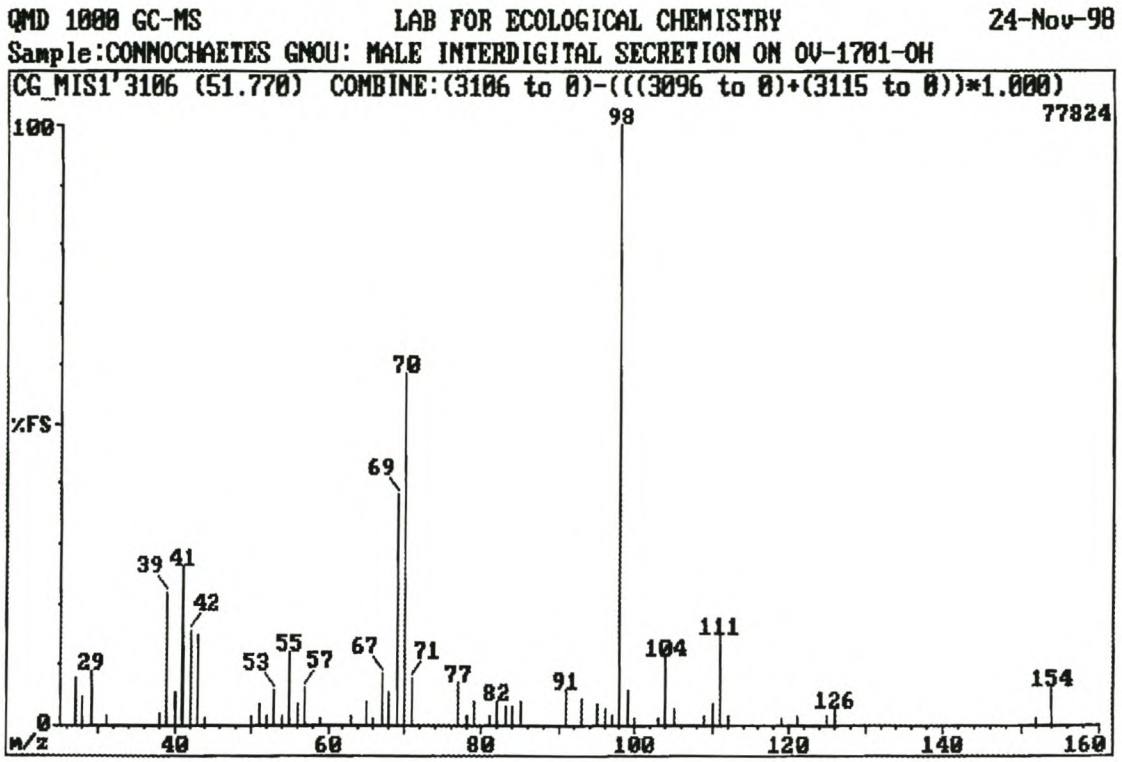


Fig. 2.61: EI mass spectrum of component 3106 (4-hydroxy-3,6,6-trimethyl-2-cyclohexen-1-one)

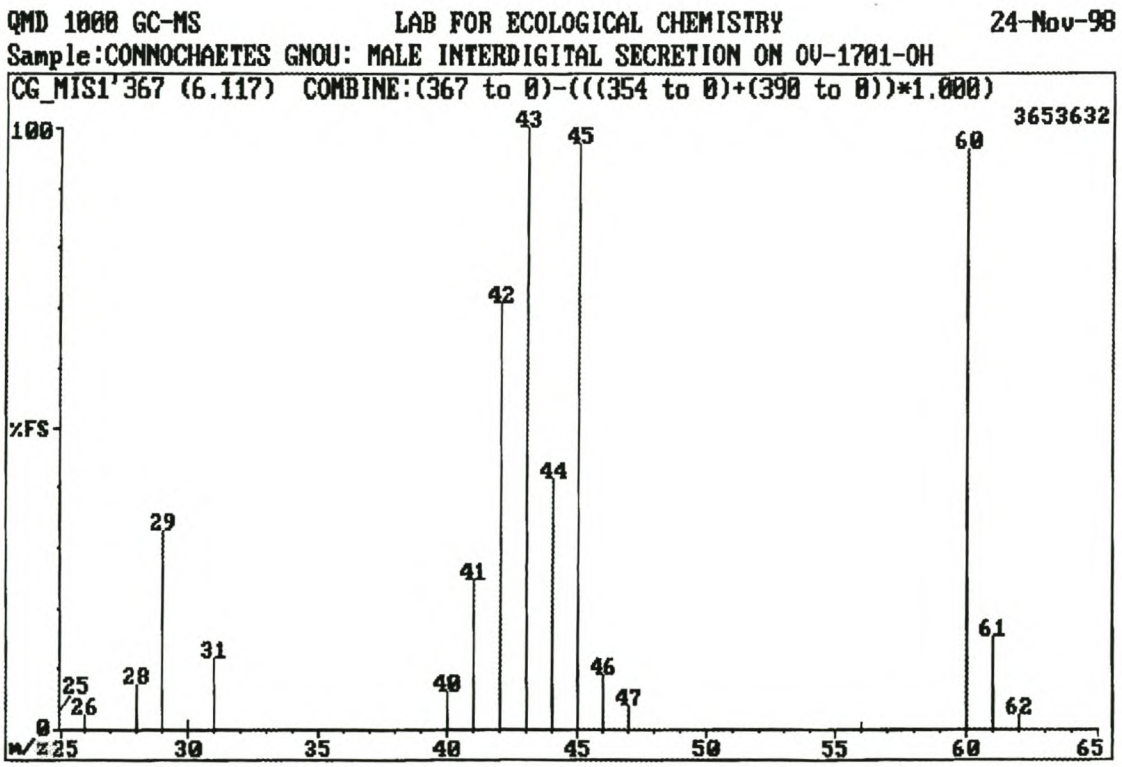


Fig. 2.62: EI mass spectrum of component 367 (acetic acid)

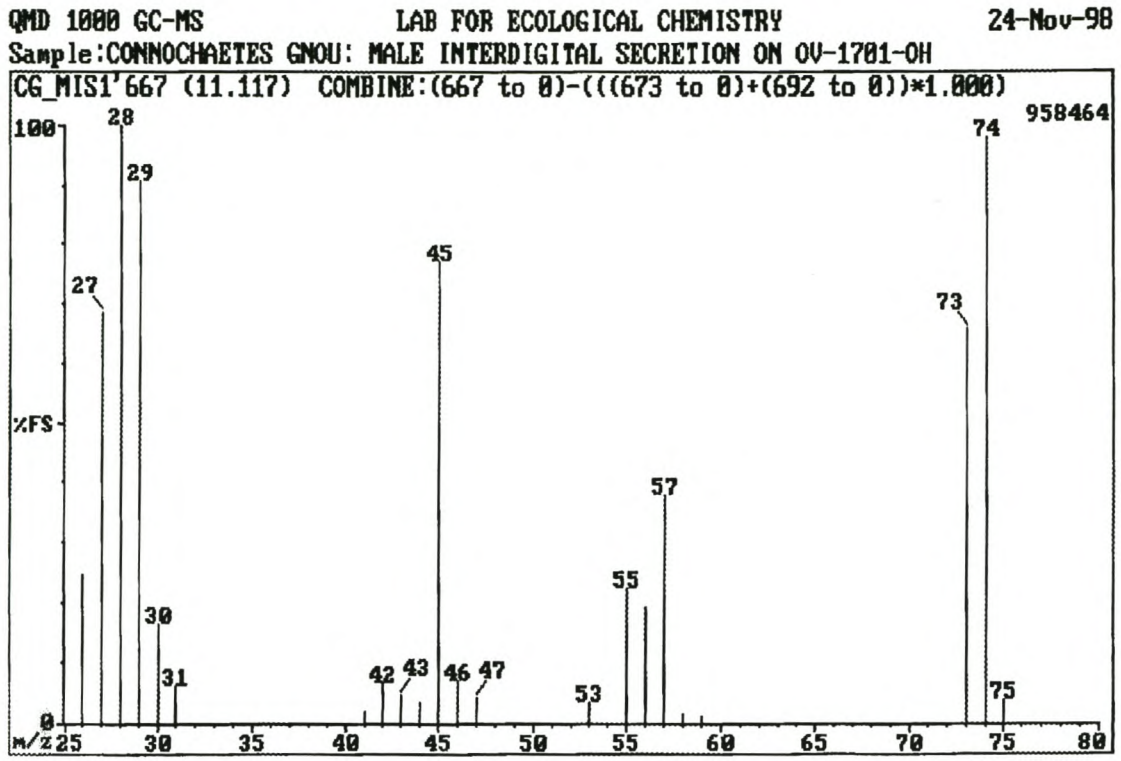


Fig. 2.63: EI mass spectrum of component 667 (propanoic acid)

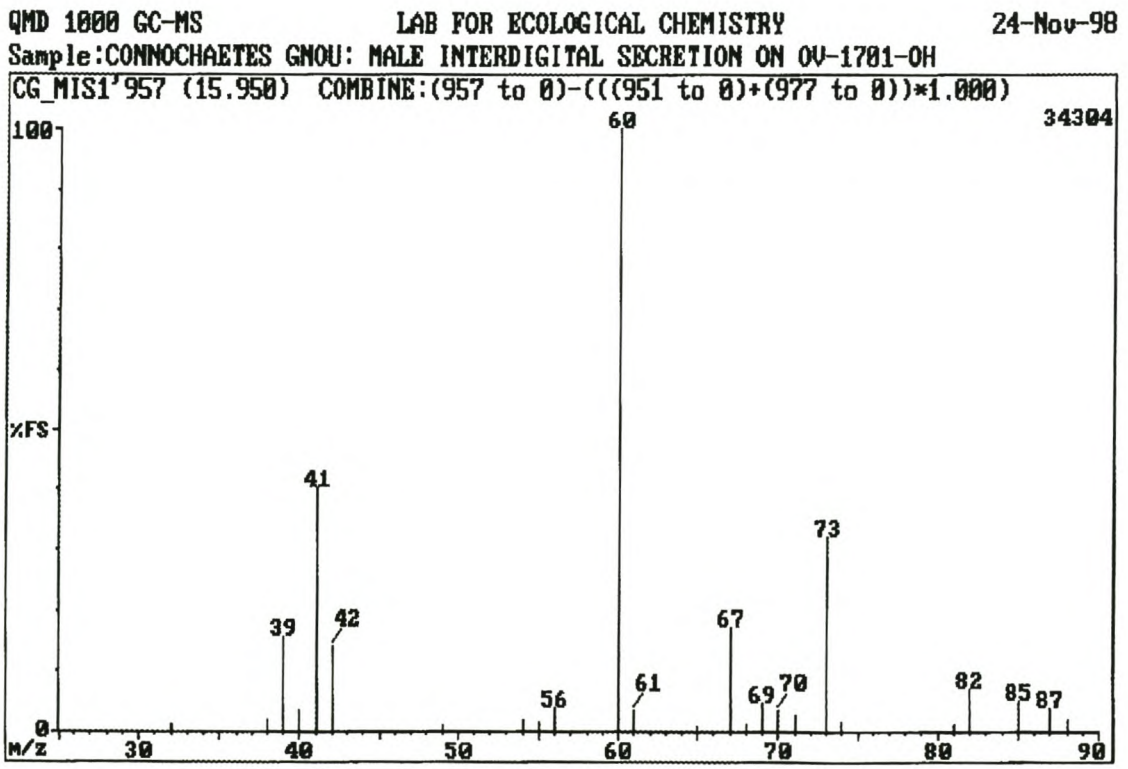


Fig. 2.64: EI mass spectrum of component 957 (butanoic acid)

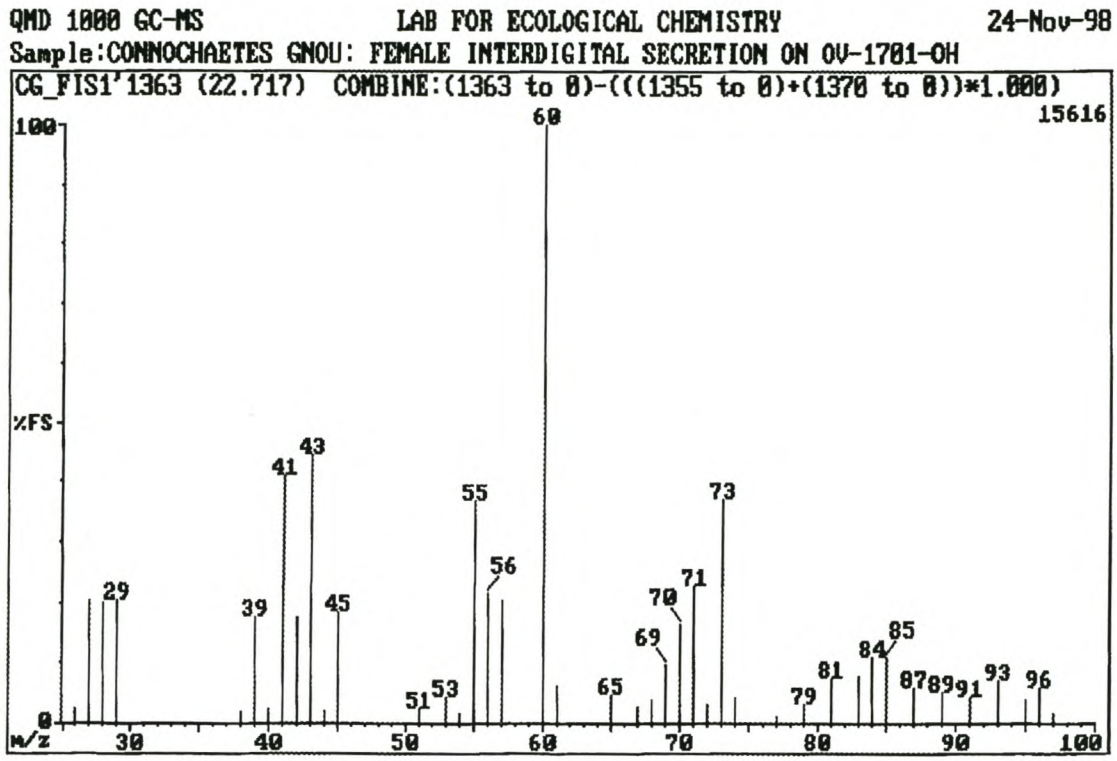


Fig. 2.65: EI mass spectrum of component 1375 (pentanoic acid)

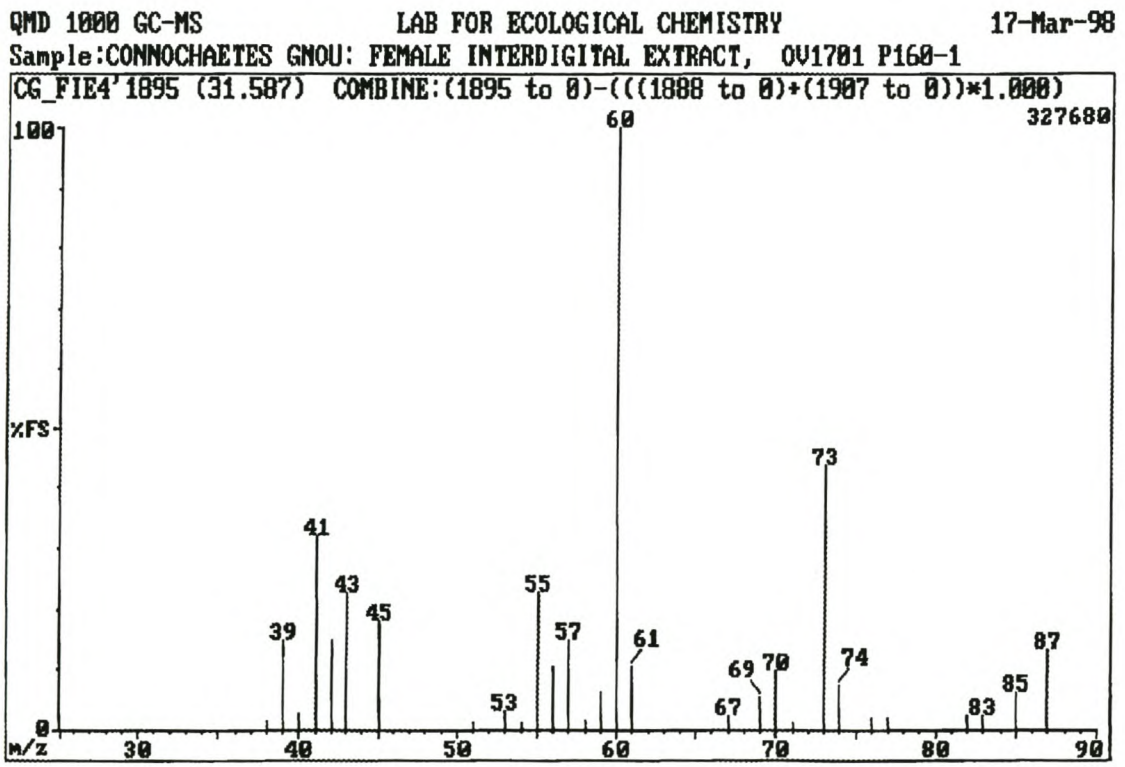


Fig. 2.66: EI mass spectrum of component 1807 (hexanoic acid)

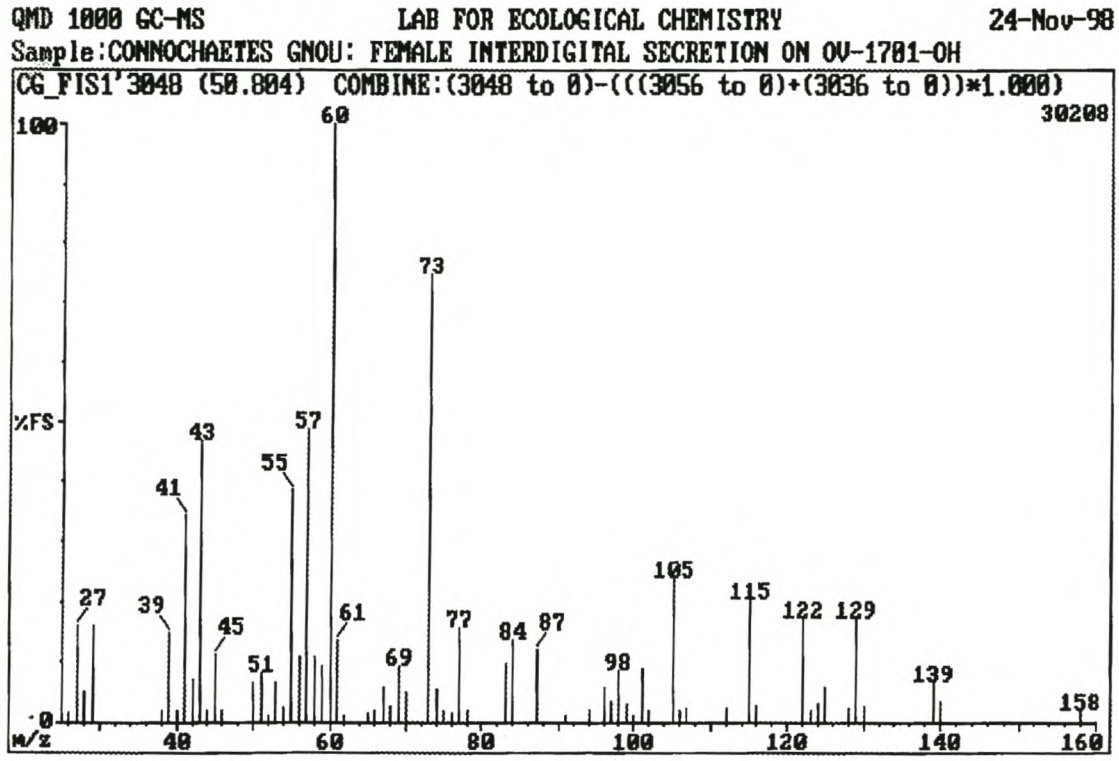


Fig. 2.67: EI mass spectrum of component 3052 (nonanoic acid)

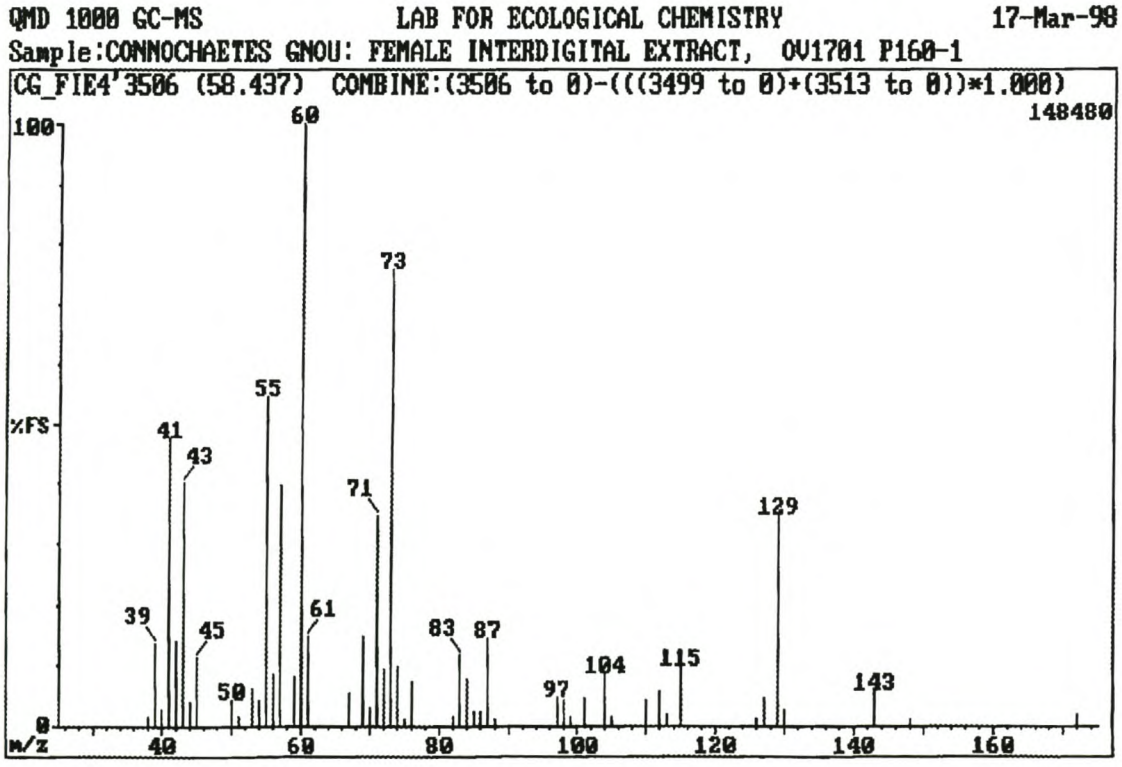


Fig. 2.68: EI mass spectrum of component 3432 (decanoic acid)

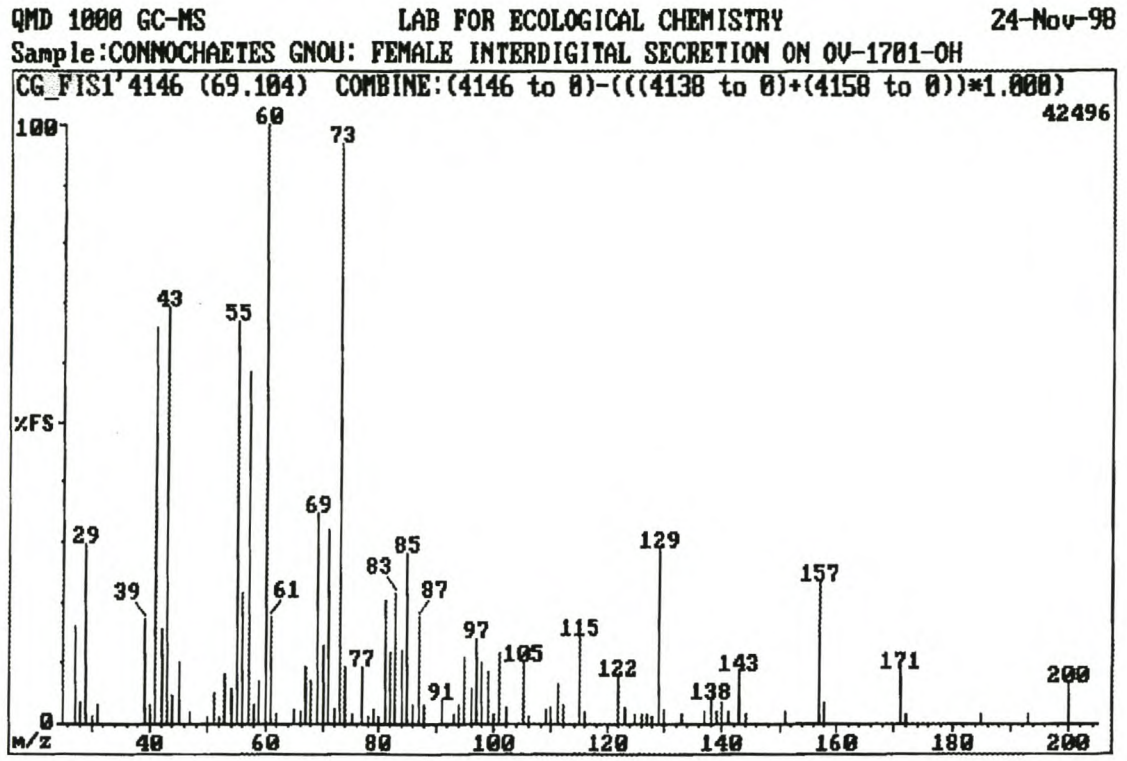


Fig. 2.69: EI mass spectrum of component 4154 (dodecanoic acid)

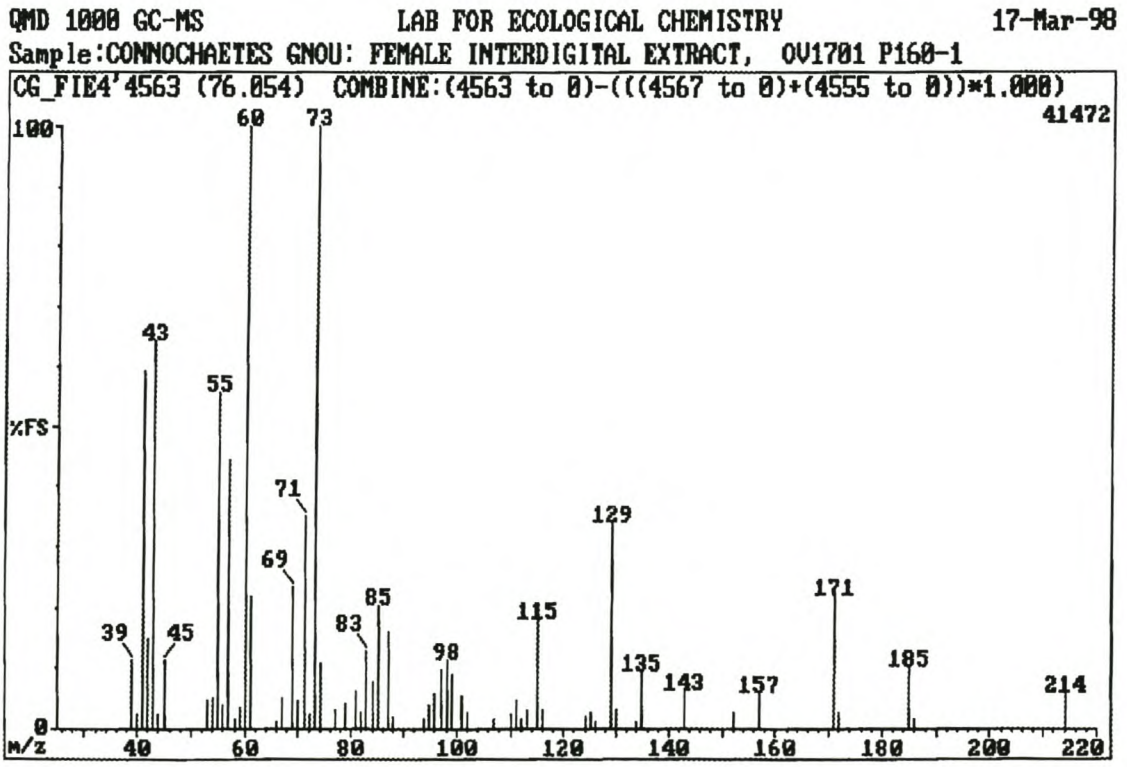


Fig. 2.70: EI mass spectrum of component 4494 (tridecanoic acid)

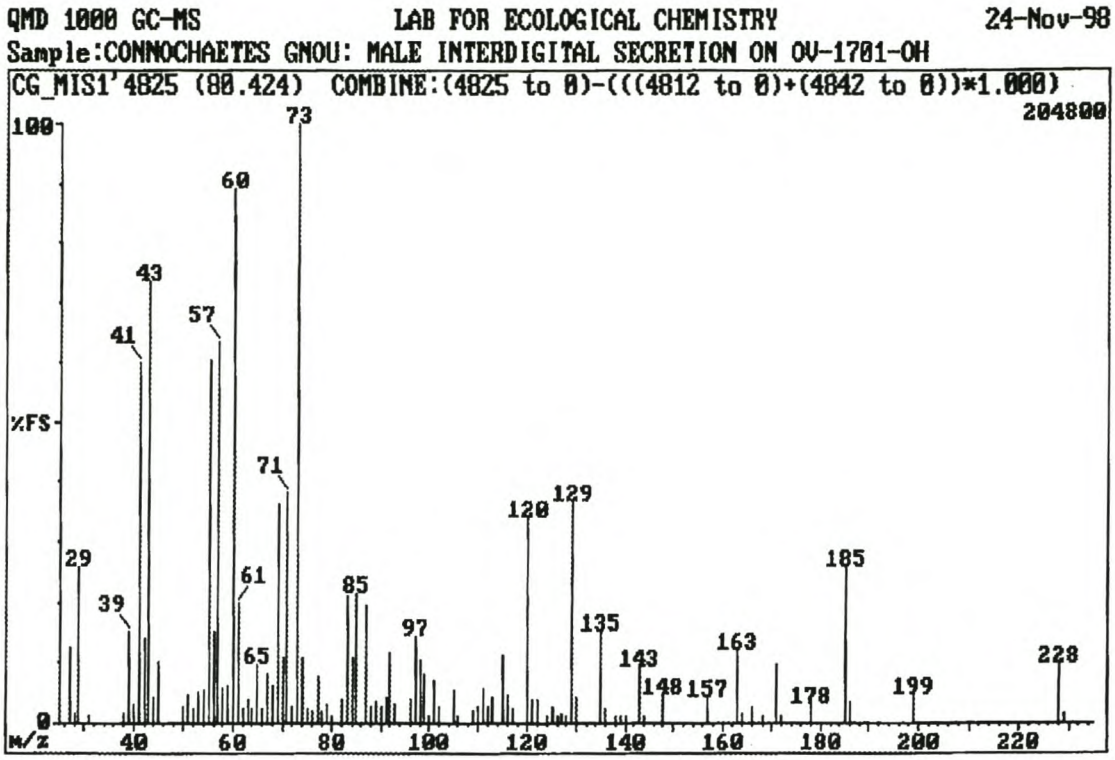


Fig. 2.71: EI mass spectrum of component 4825 (tetradecanoic acid)

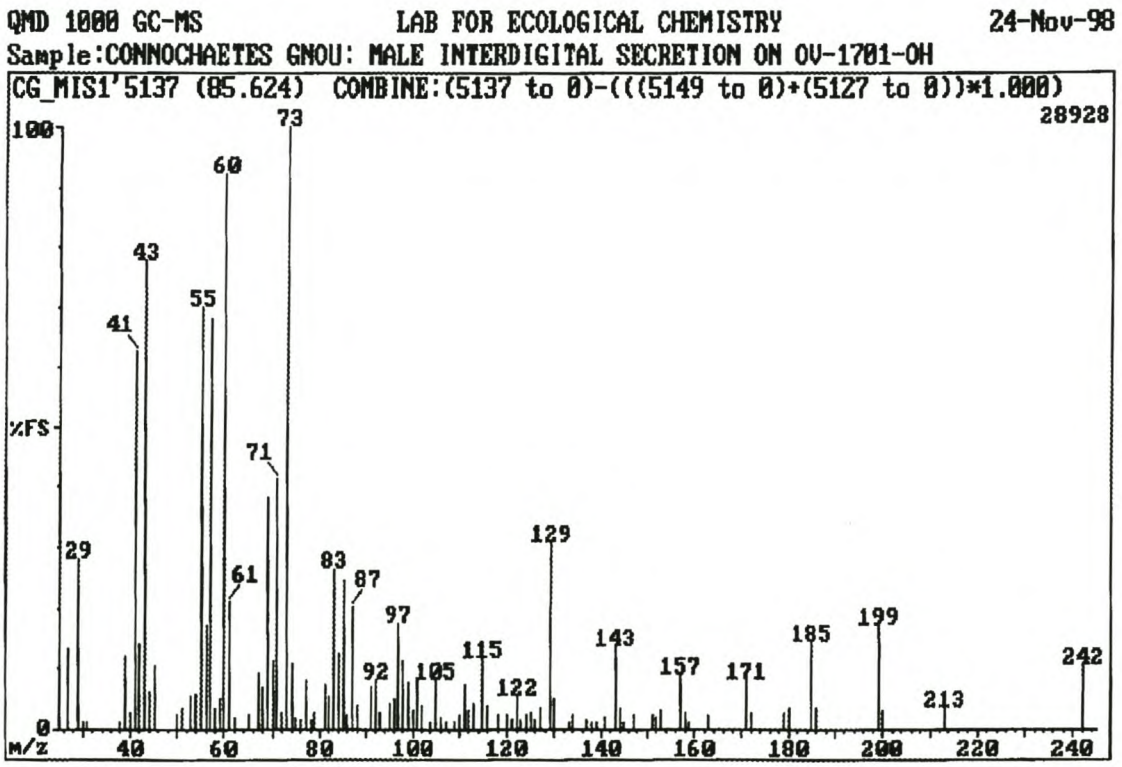


Fig. 2.72: EI mass spectrum of component 5137 (pentadecanoic acid)

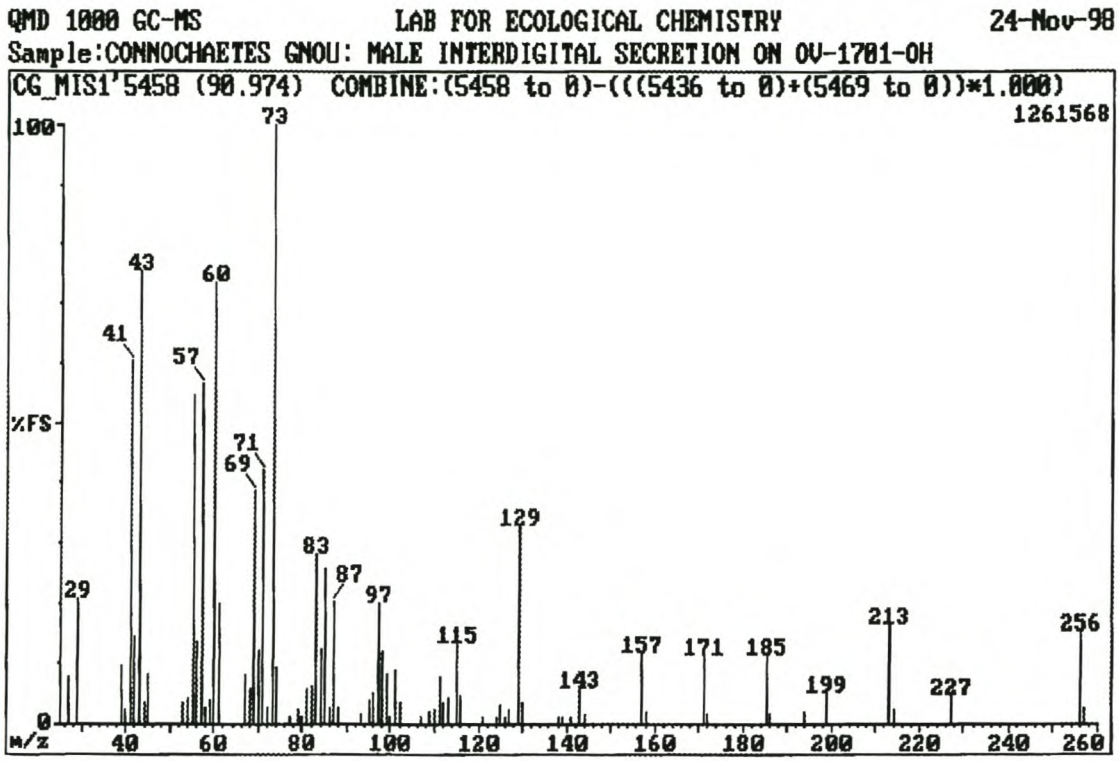


Fig. 2.73: EI mass spectrum of component 5458 (hexadecanoic acid)

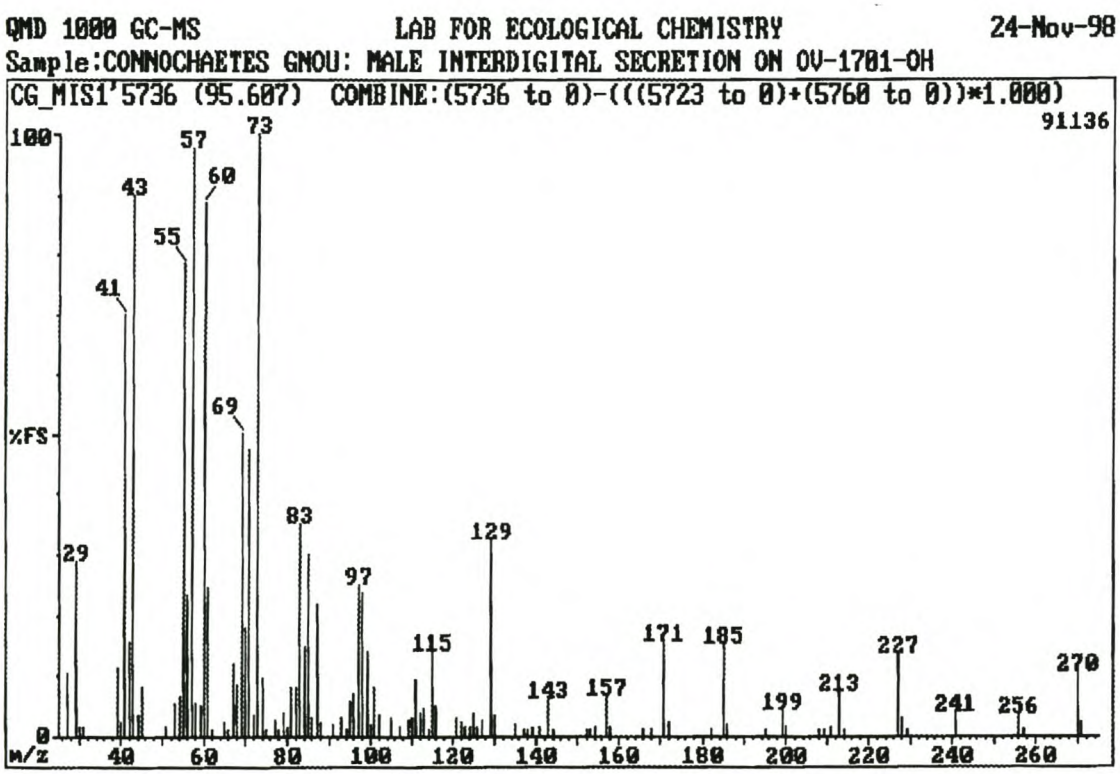


Fig. 2.74: EI mass spectrum of component 5736 (heptadecanoic acid)

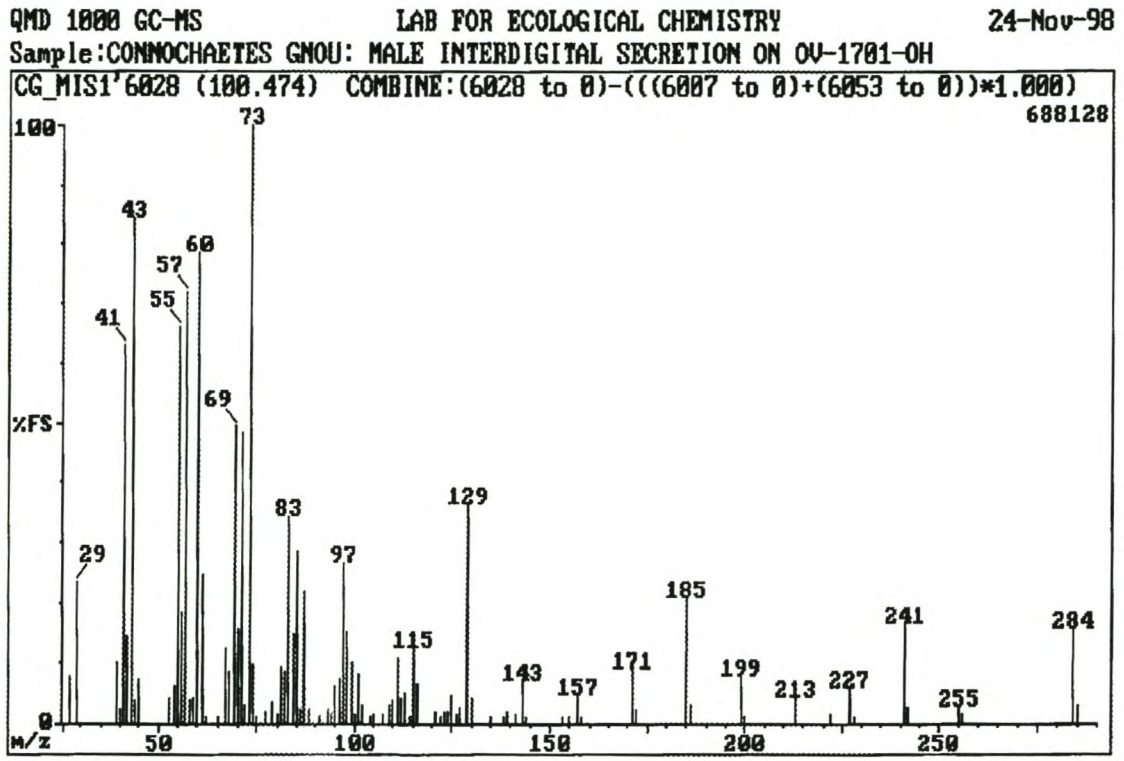


Fig. 2.75: EI mass spectrum of component 6028 (octadecanoic acid)

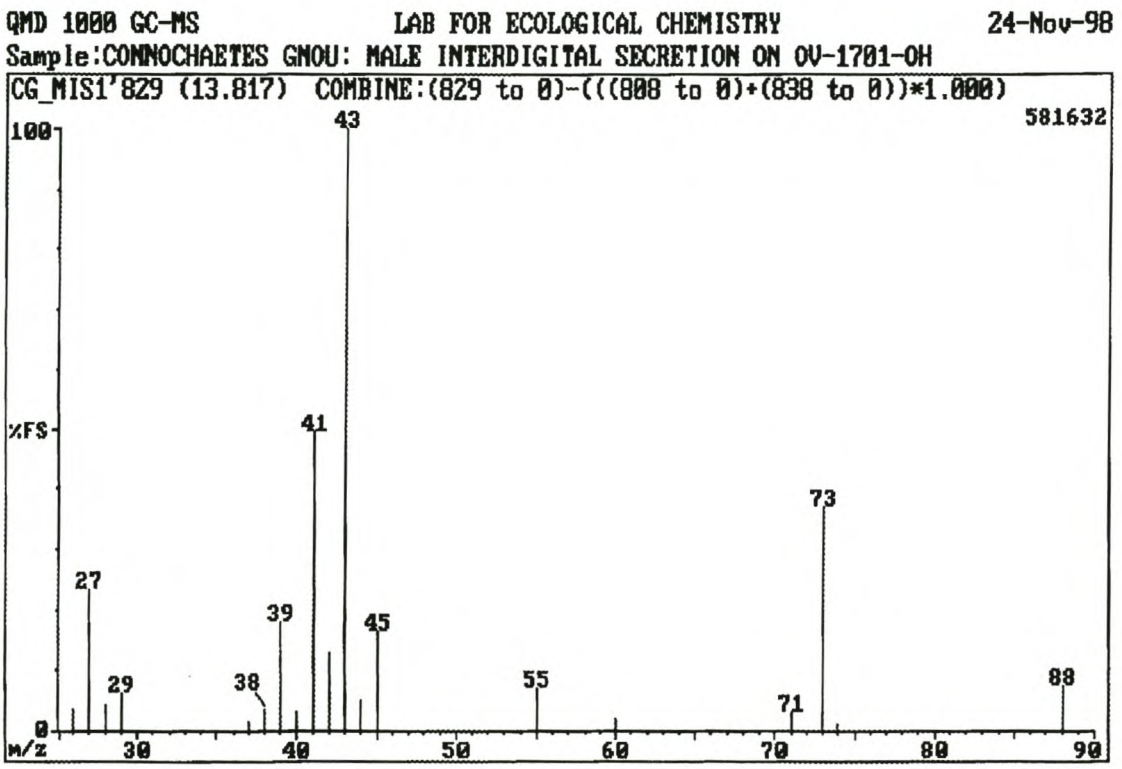


Fig. 2.76: EI mass spectrum of component 829 (2-methylpropanoic acid)

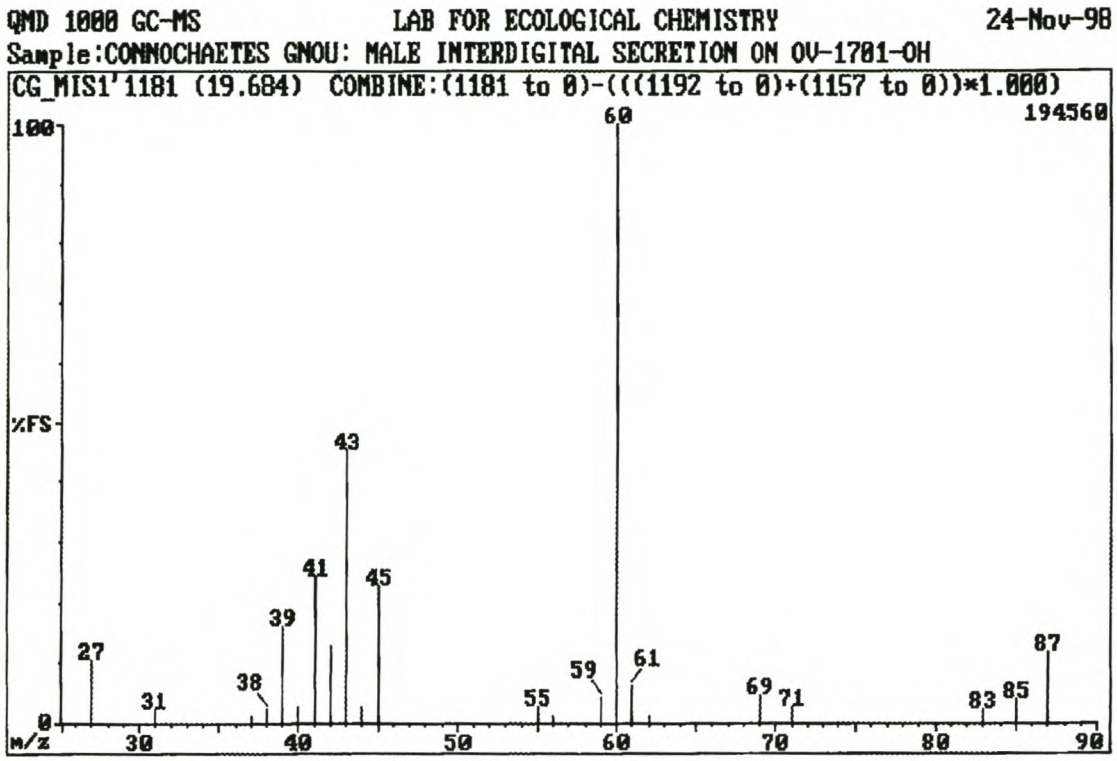


Fig. 2.77: EI mass spectrum of component 1181 (3-methylbutanoic acid)

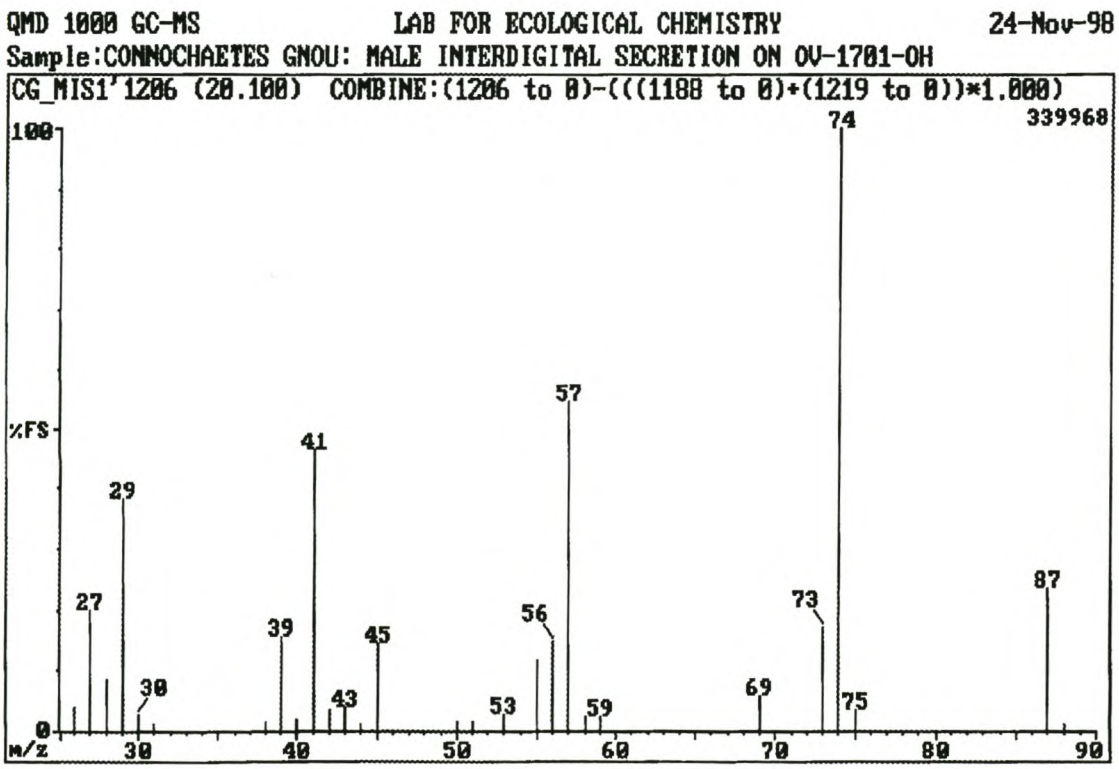


Fig. 2.78: EI mass spectrum of component 1206 (2-methylbutanoic acid)

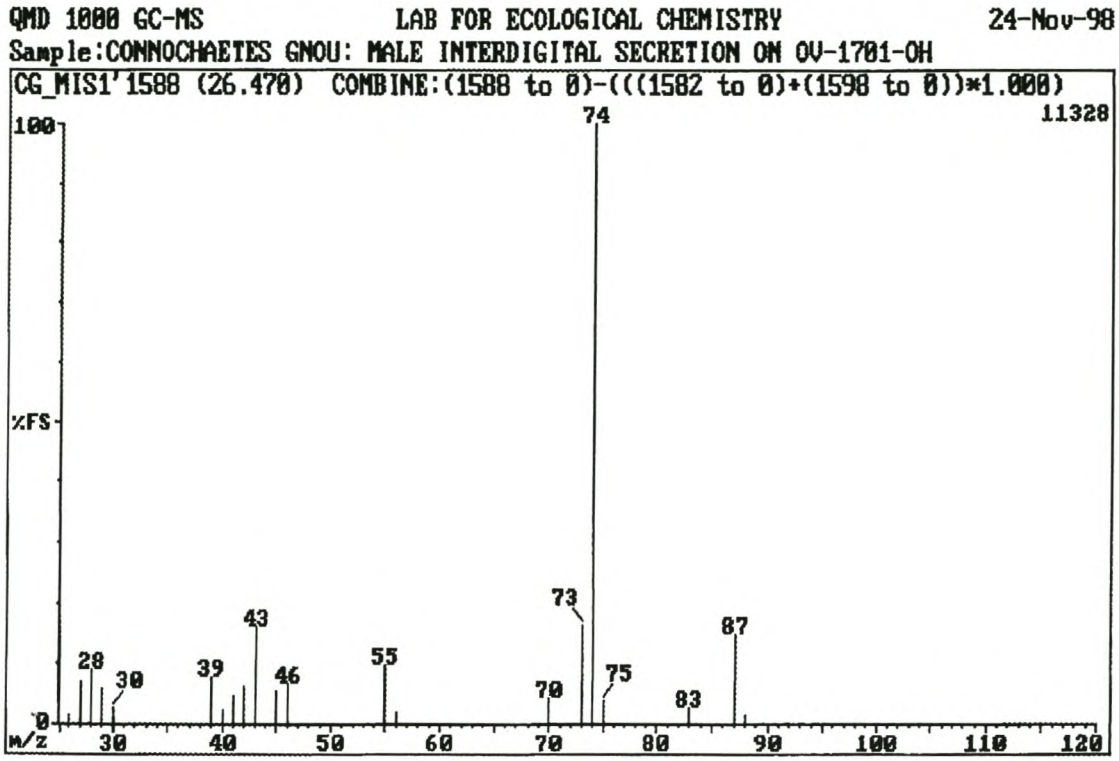


Fig. 2.79: EI mass spectrum of component 1588 (2-methylpentanoic acid)

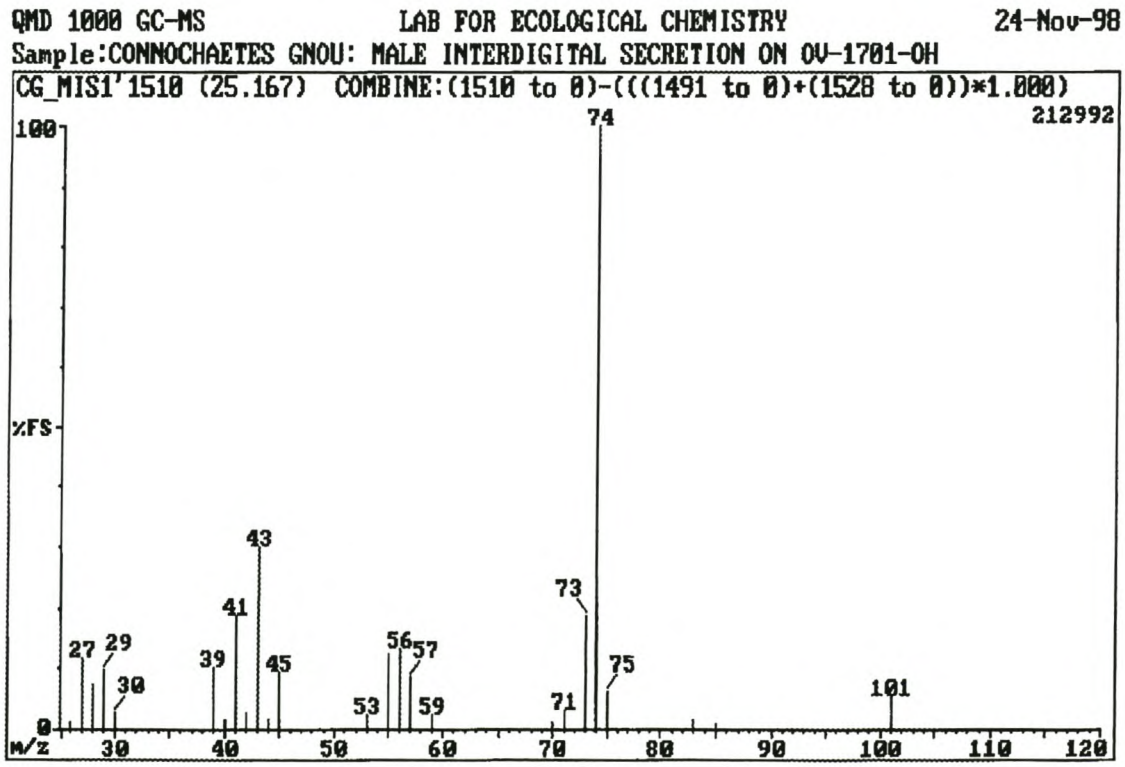


Fig. 2.80: EI mass spectrum of component 1510 (2,3-dimethylbutanoic acid)

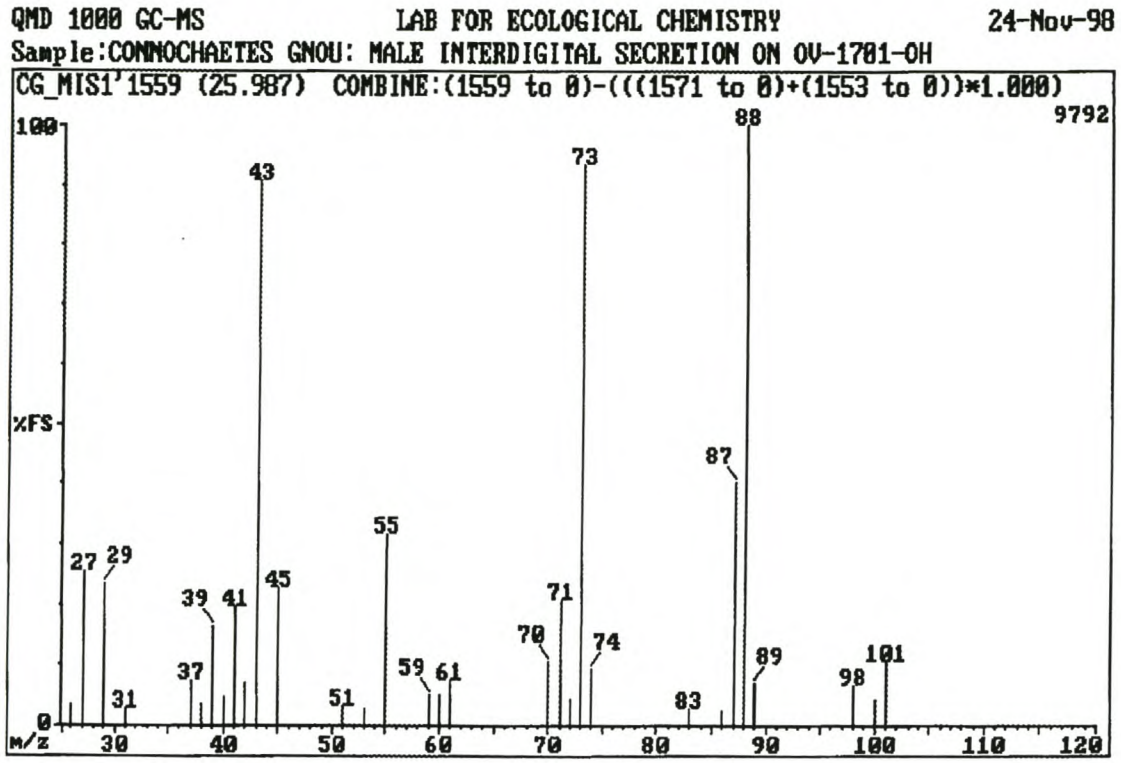


Fig. 2.81: EI mass spectrum of component 1559 (2-ethylbutanoic acid)

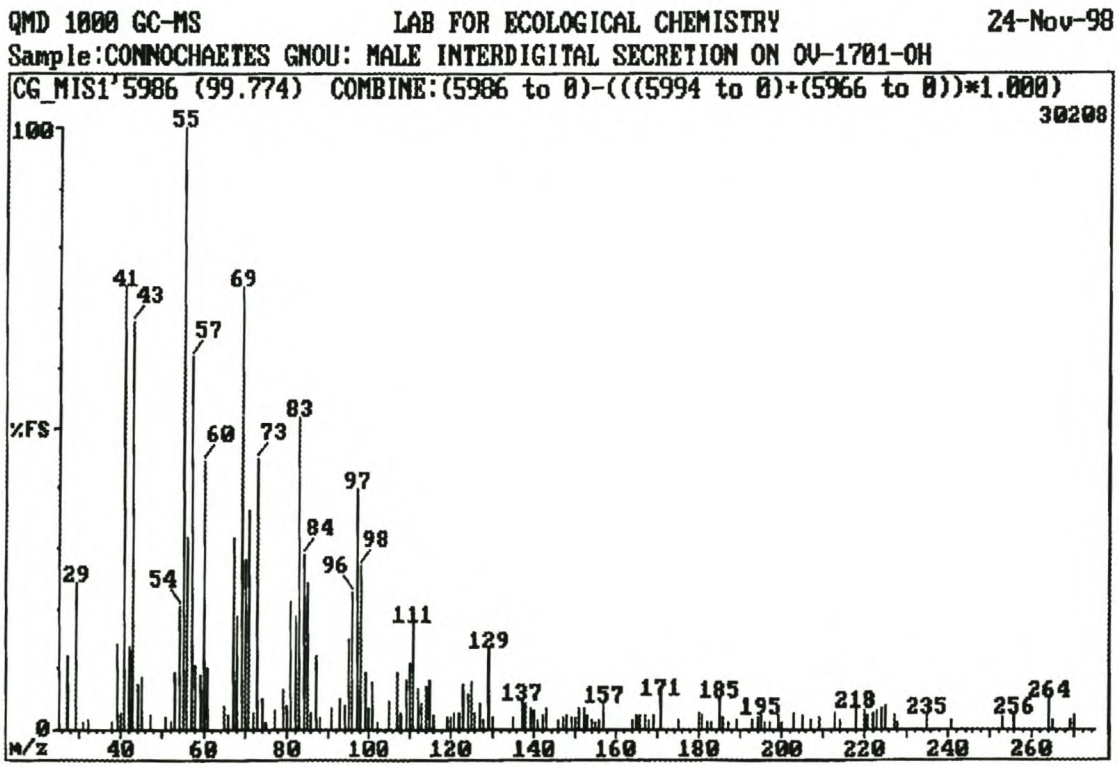


Fig. 2.82: EI mass spectrum of component 5986 [(*Z*)-9-octadecenoic acid]

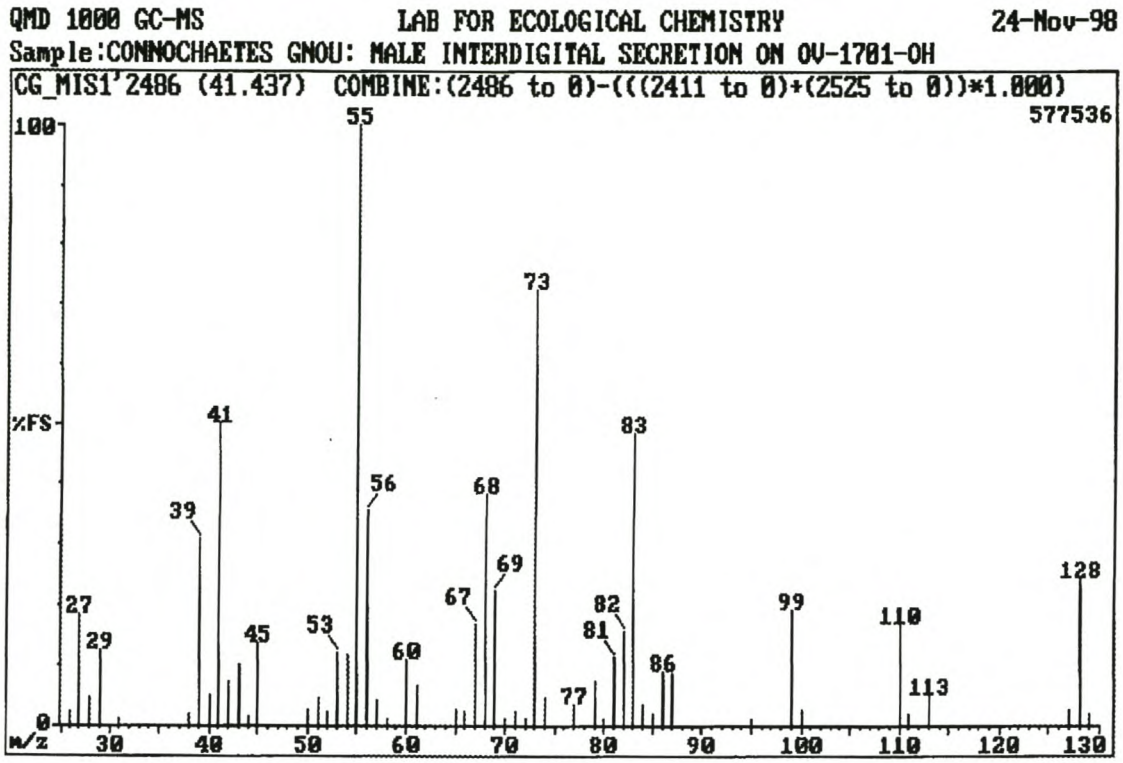


Fig. 2.83: EI mass spectrum of component 2486 (cyclohexanecarboxylic acid)

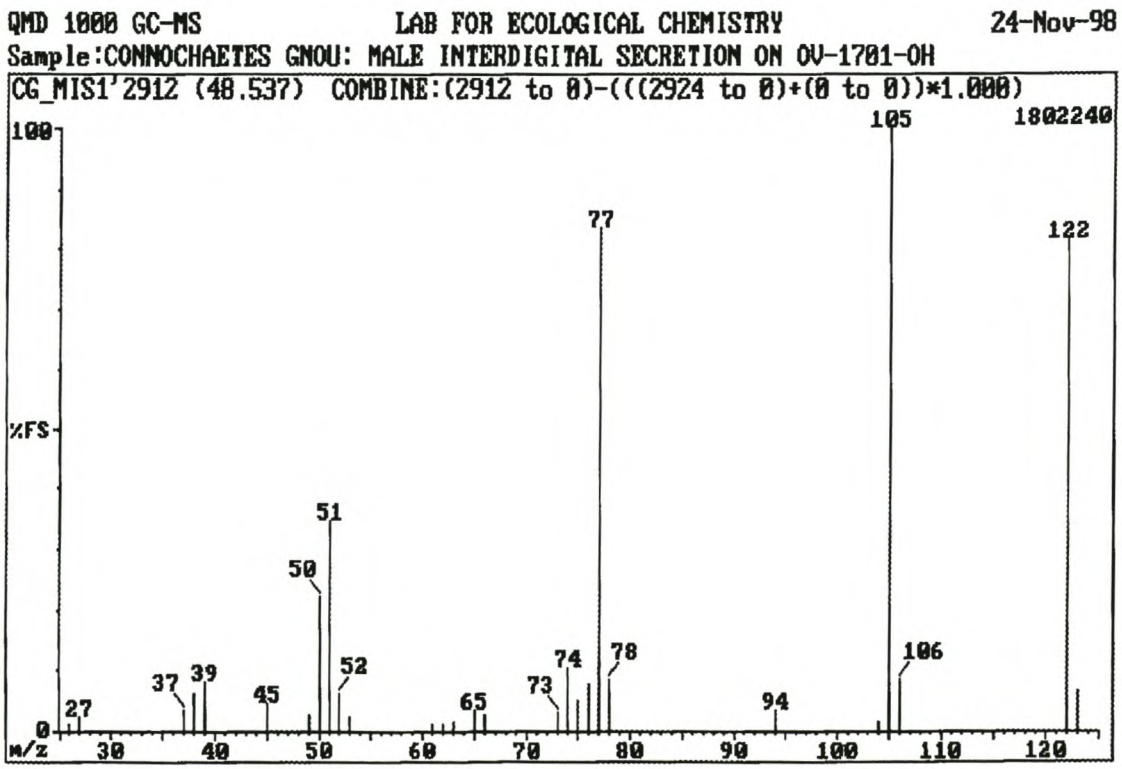


Fig. 2.84: EI mass spectrum of component 2912 (benzoic acid)

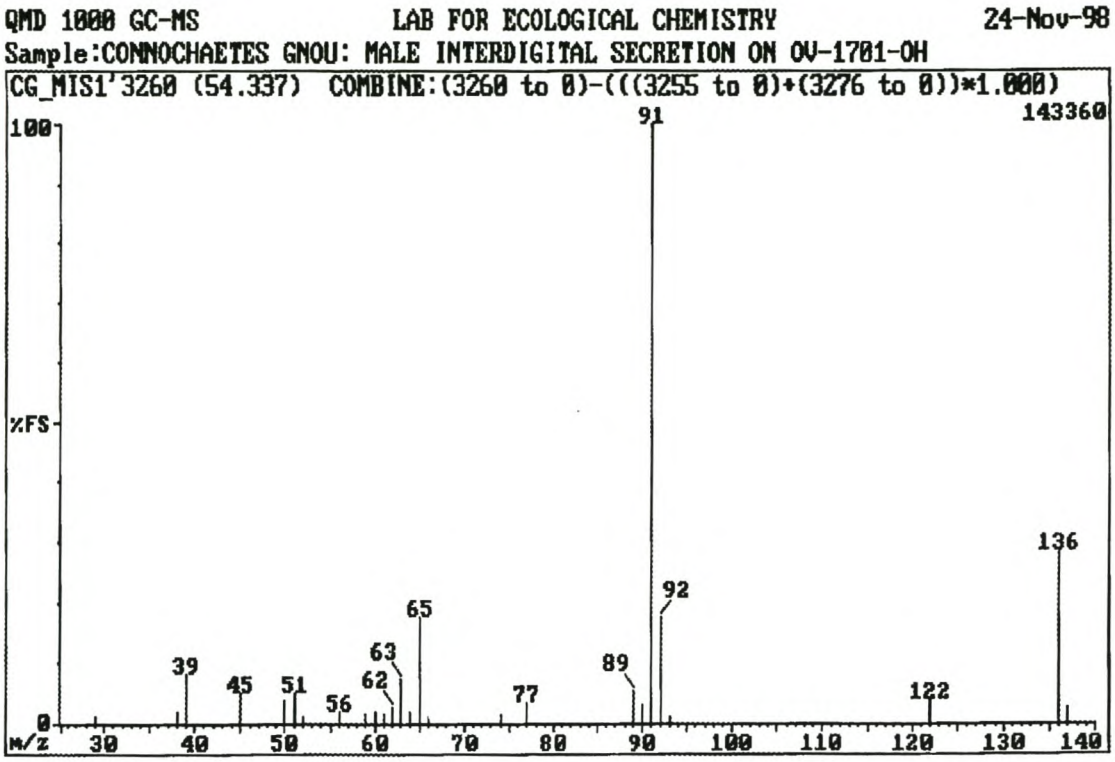


Fig. 2.85: EI mass spectrum of component 3260 (phenylacetic acid)

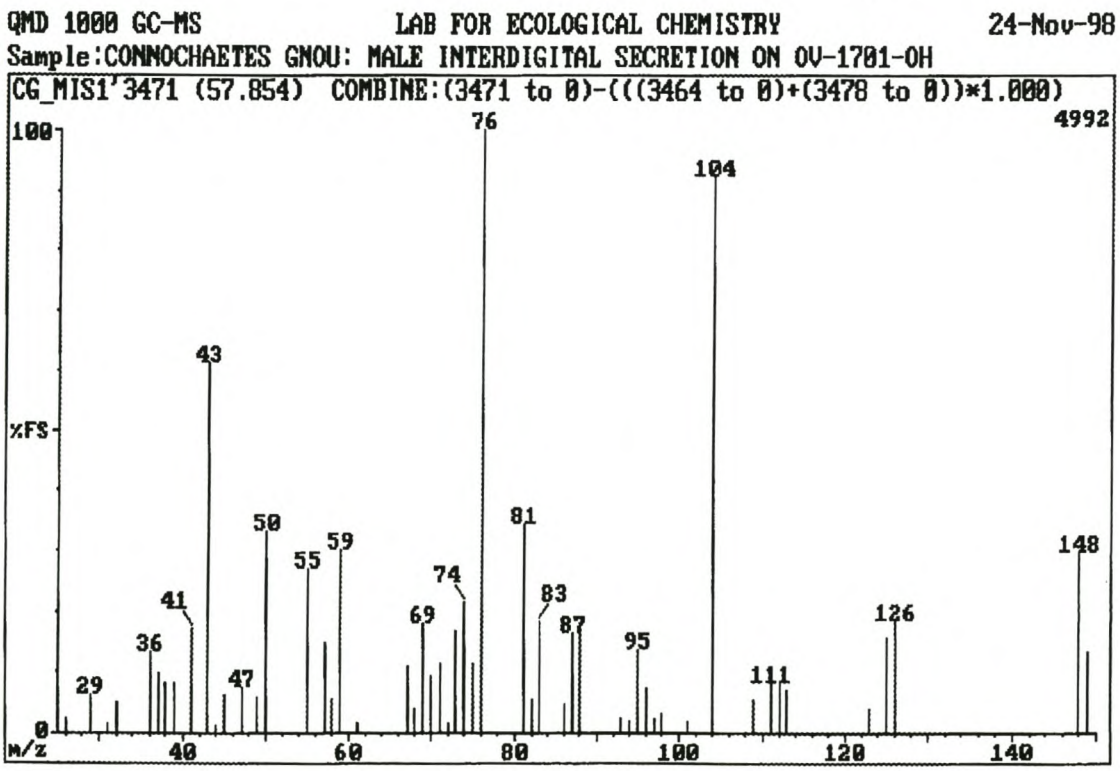


Fig. 2.86: EI mass spectrum of component 3471 (phthalic anhydride)

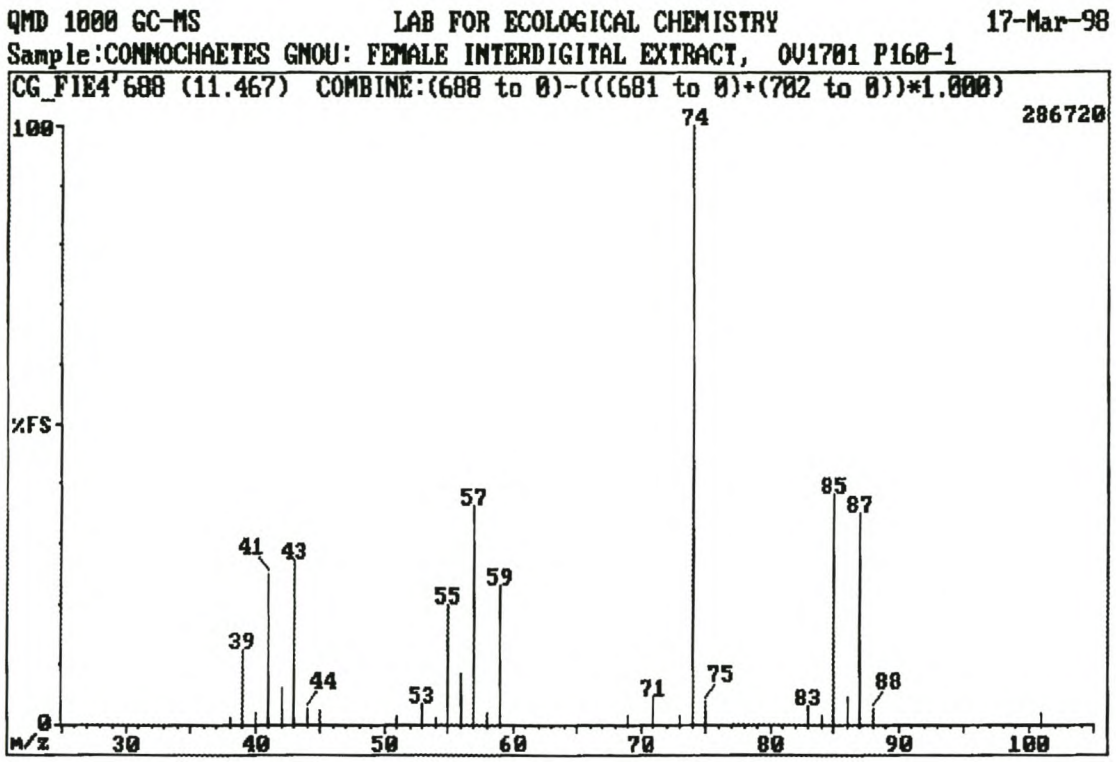


Fig. 2.87: EI mass spectrum of component 655 (methyl pentanoate)

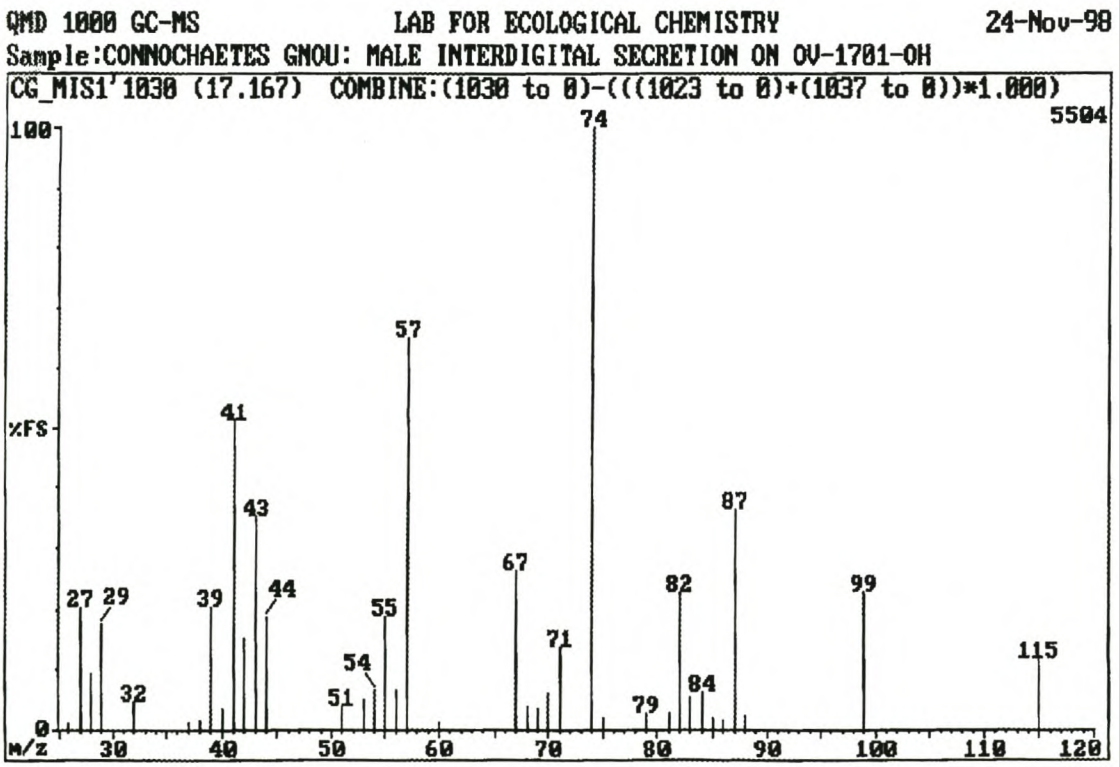


Fig. 2.88: EI mass spectrum of component 1030 (methyl hexanoate)

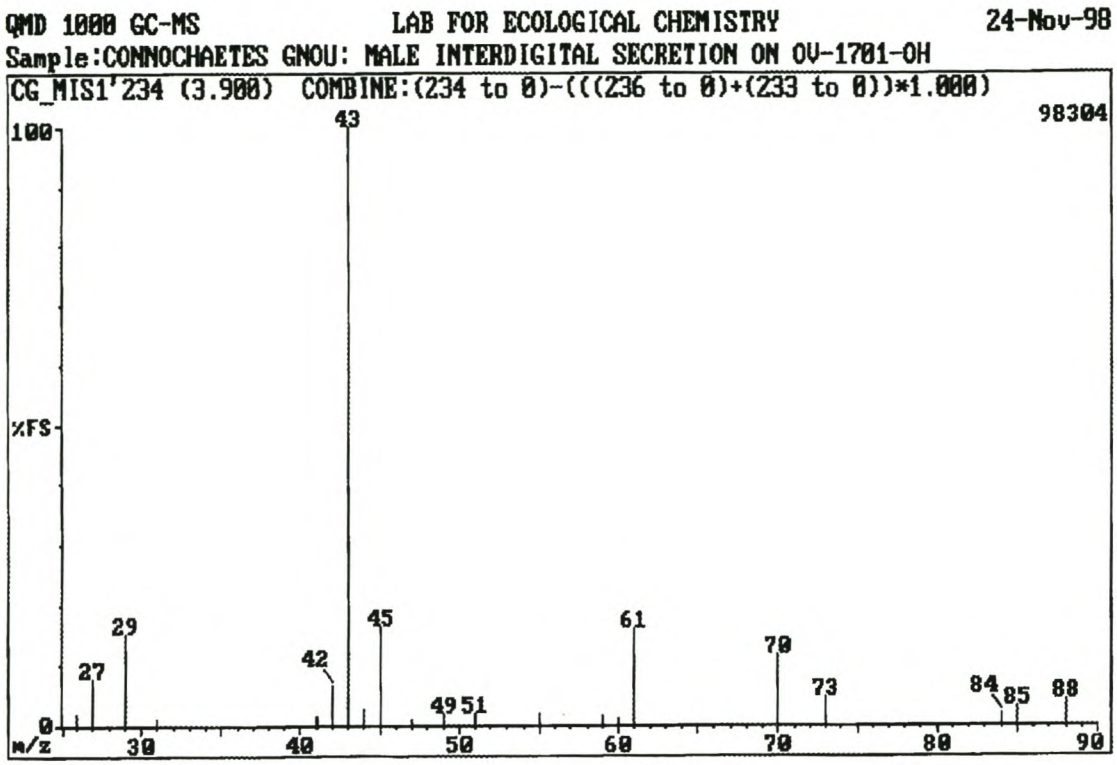


Fig. 2.89: EI mass spectrum of component 234 (ethyl acetate)

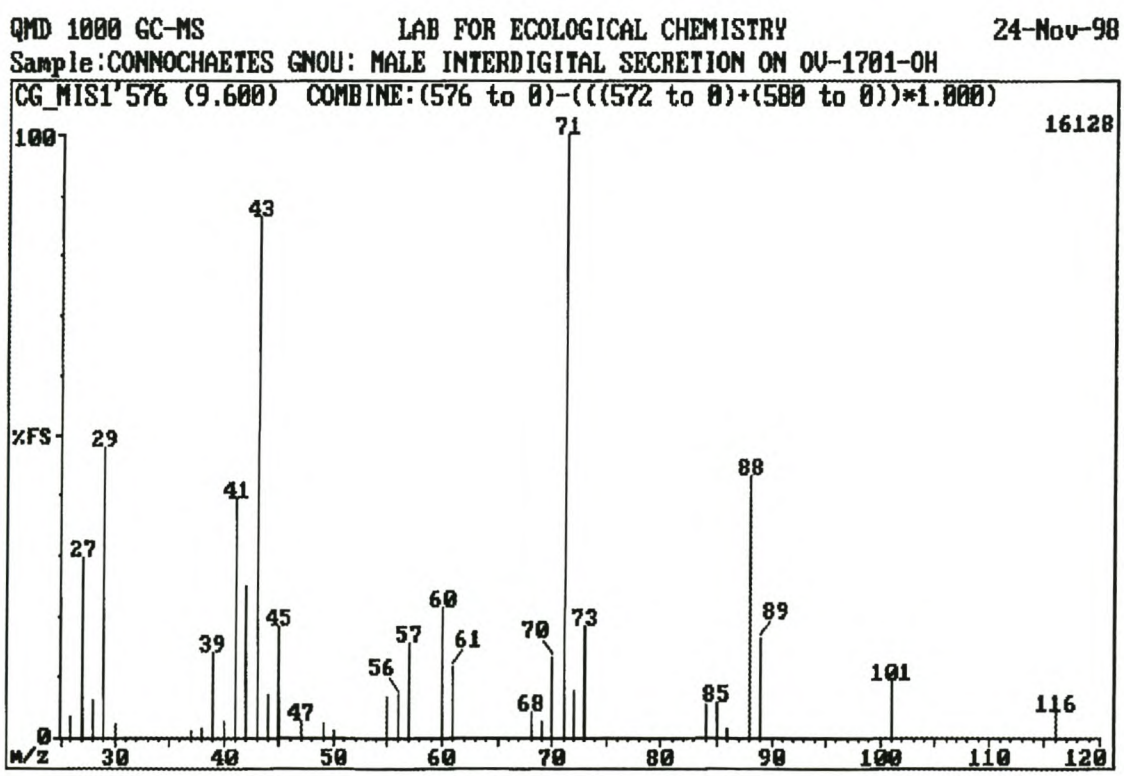


Fig. 2.90: EI mass spectrum of component 576 (ethyl butanoate)

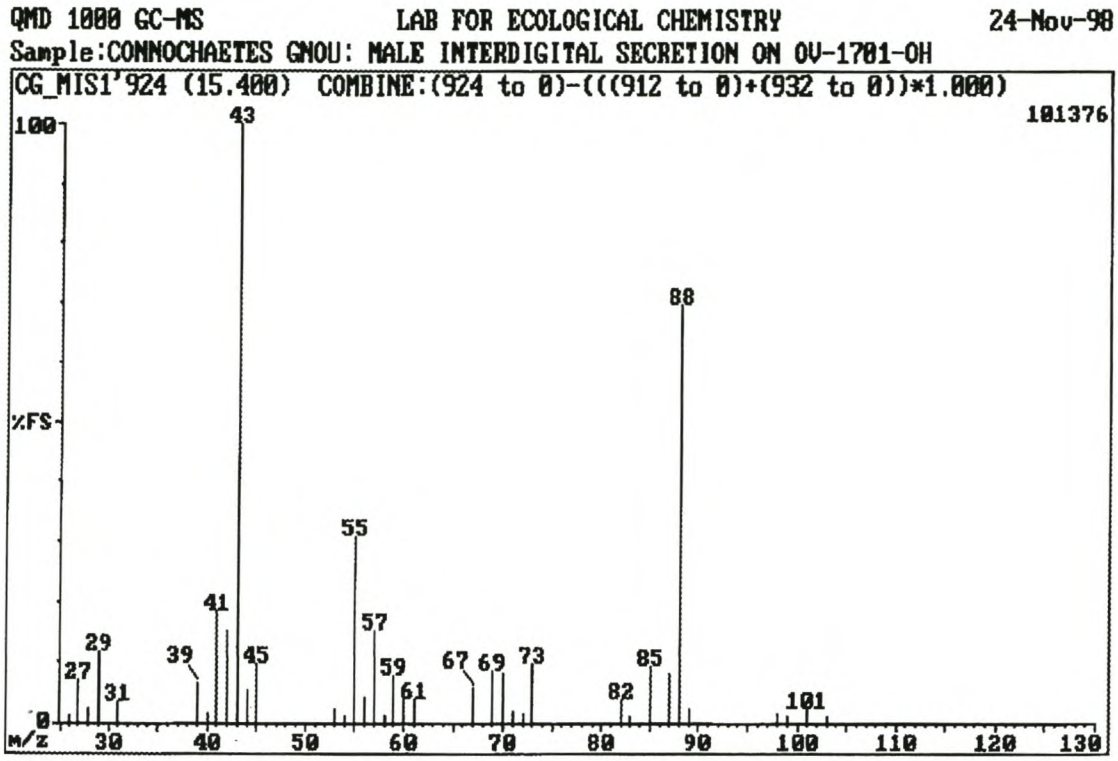


Fig. 2.91: EI mass spectrum of component 924 (ethyl pentanoate)

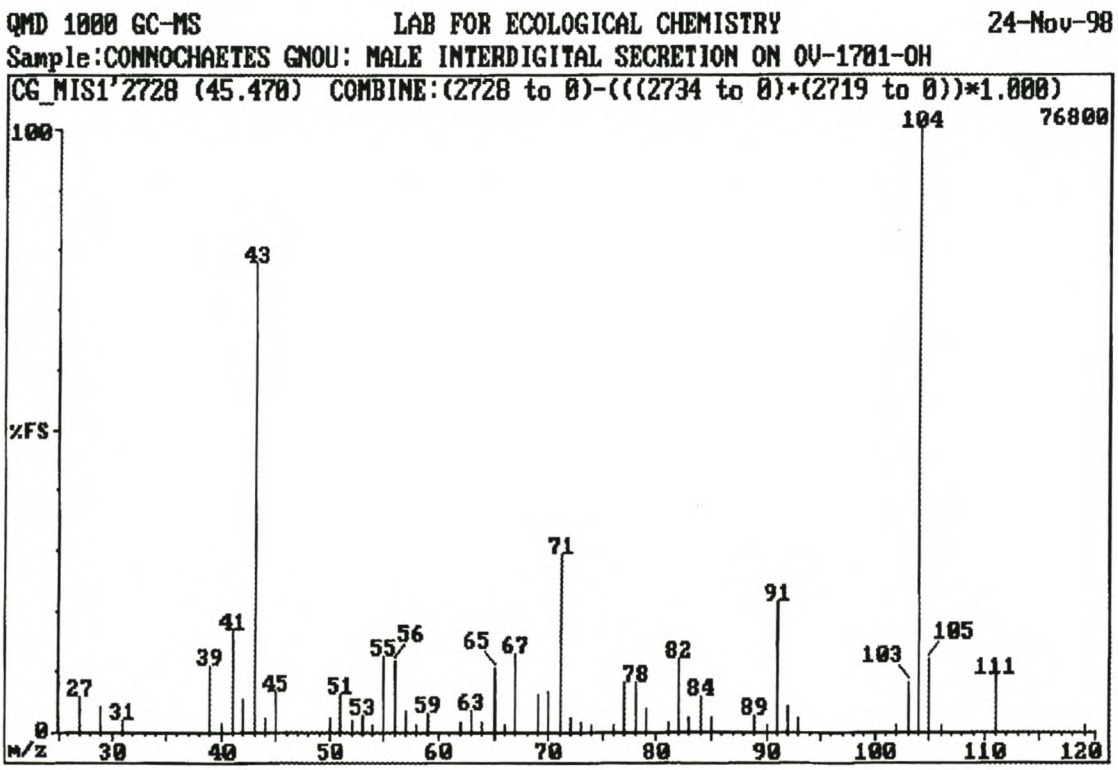


Fig. 2.92: EI mass spectrum of component 2728 (2-phenylethyl acetate)

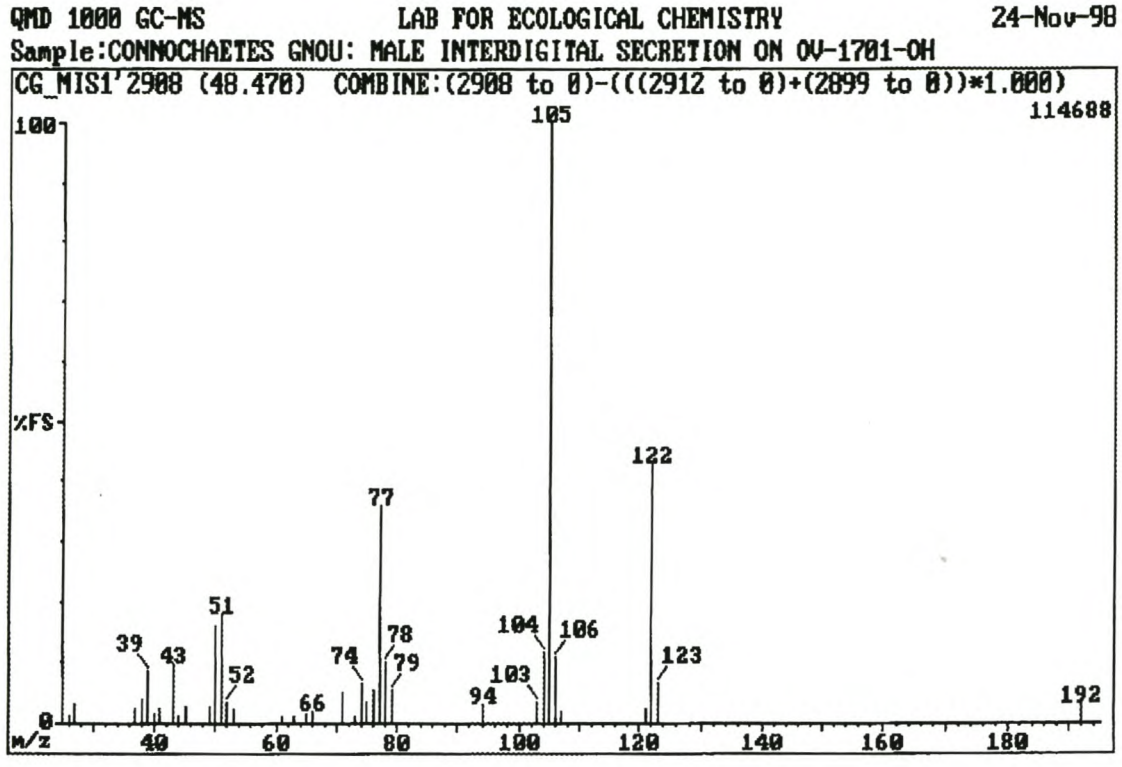


Fig. 2.93: EI mass spectrum of component 2908 (1-phenylethyl 2-methylpropanoate)

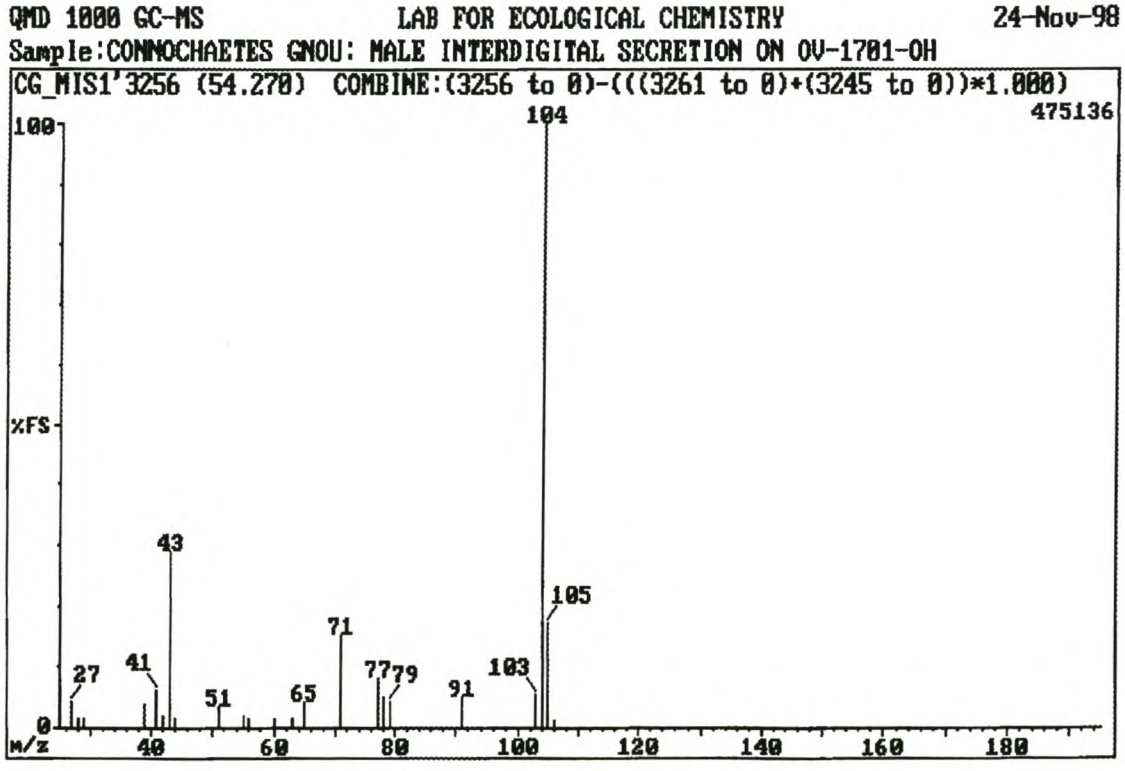


Fig. 2.94: EI mass spectrum of component 3256 (2-phenylethyl 2-methylpropanoate)

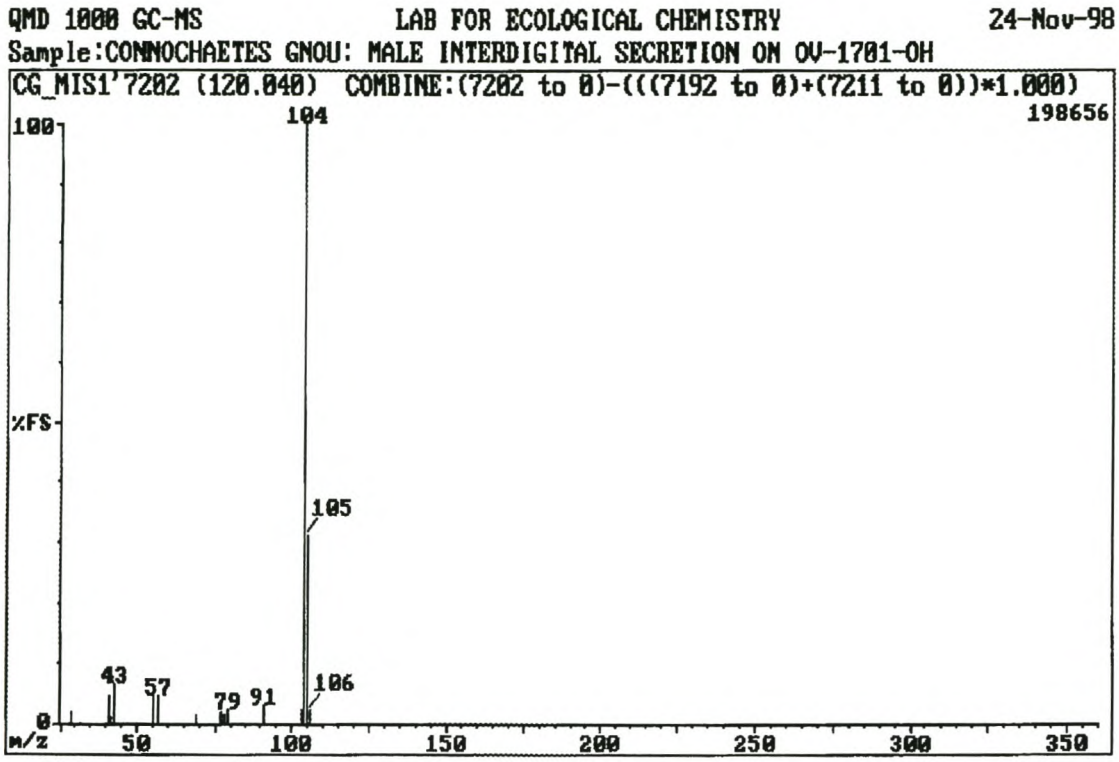


Fig. 2.95: EI mass spectrum of component 7202 (2-phenylethyl hexadecanoate)

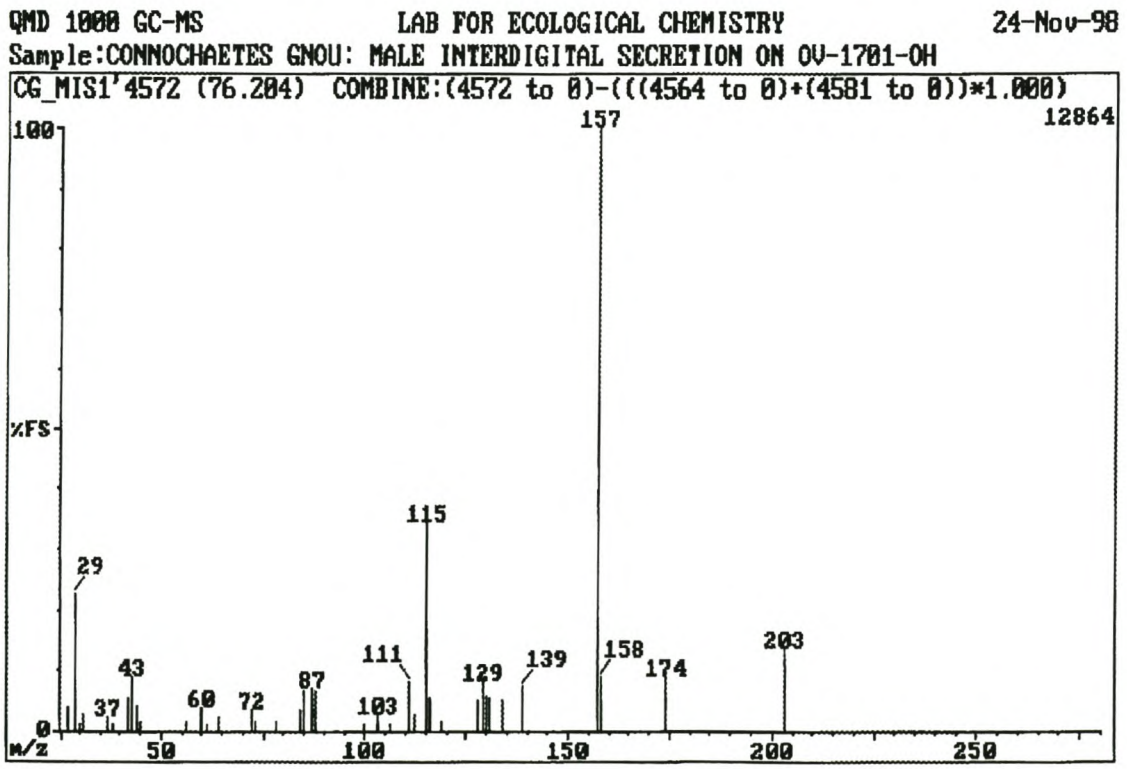


Fig. 2.96: EI mass spectrum of component 4572 (triethyl citrate)

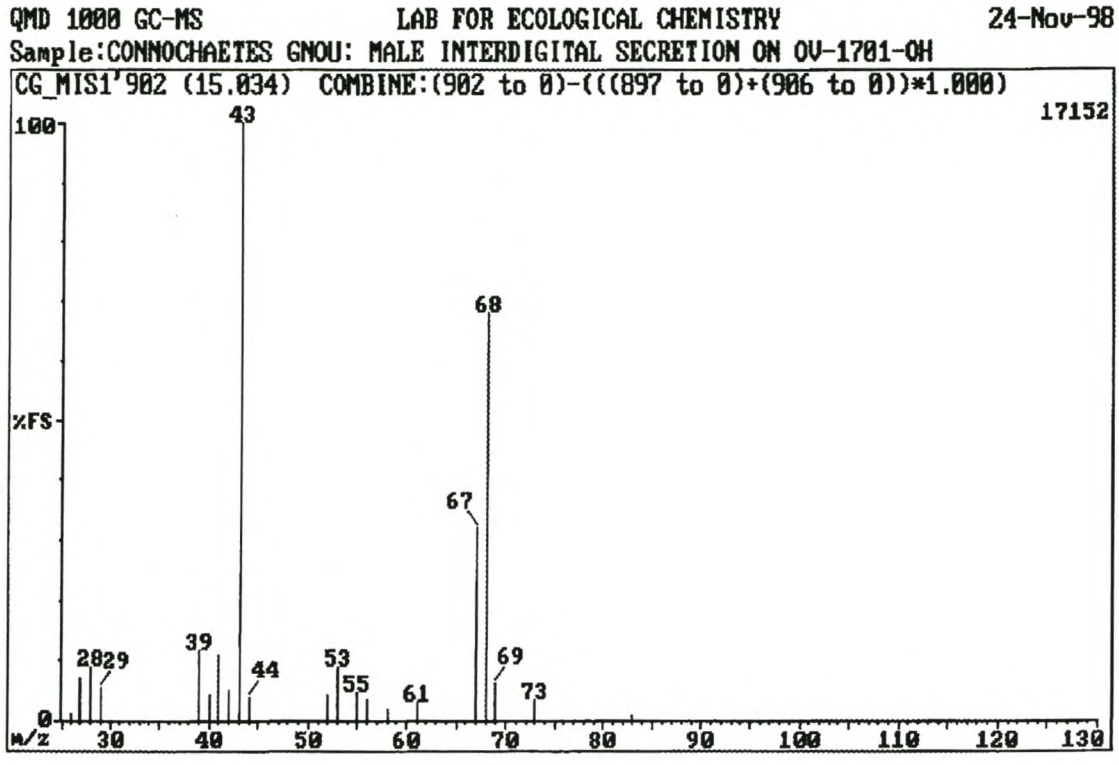


Fig. 2.97: EI mass spectrum of component 902 (3-methyl-3-butenyl acetate)

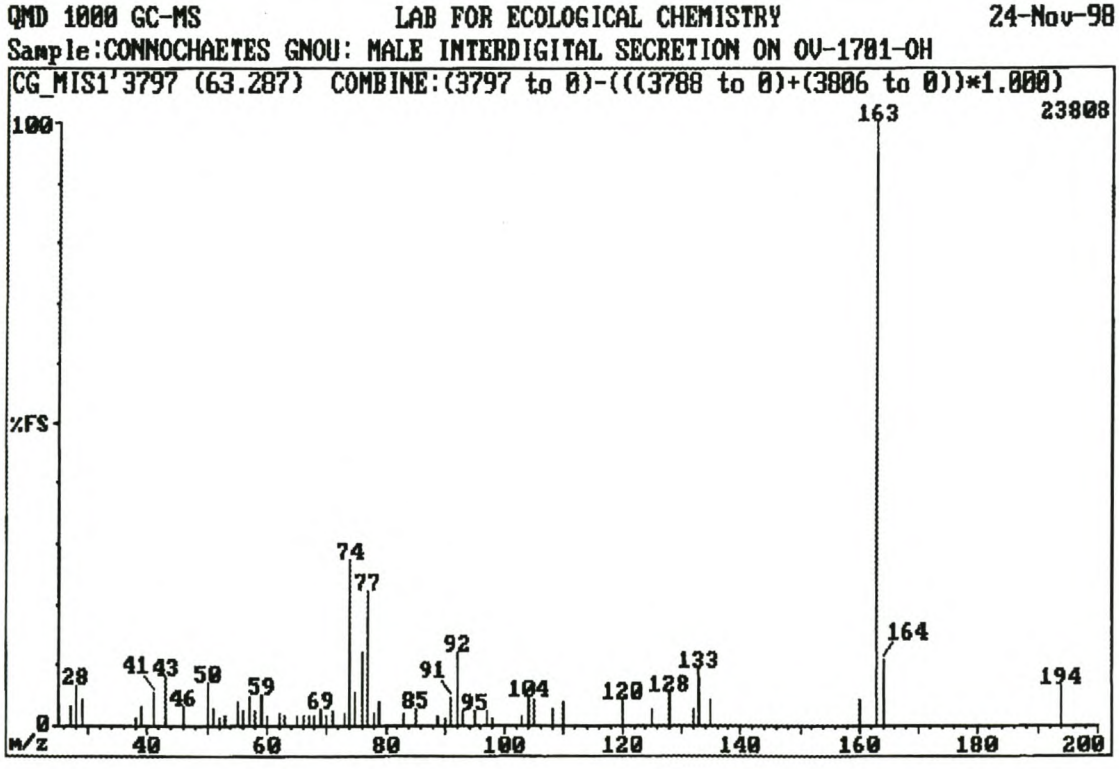


Fig. 2.98: EI mass spectrum of component 3797 (dimethyl phthalate)

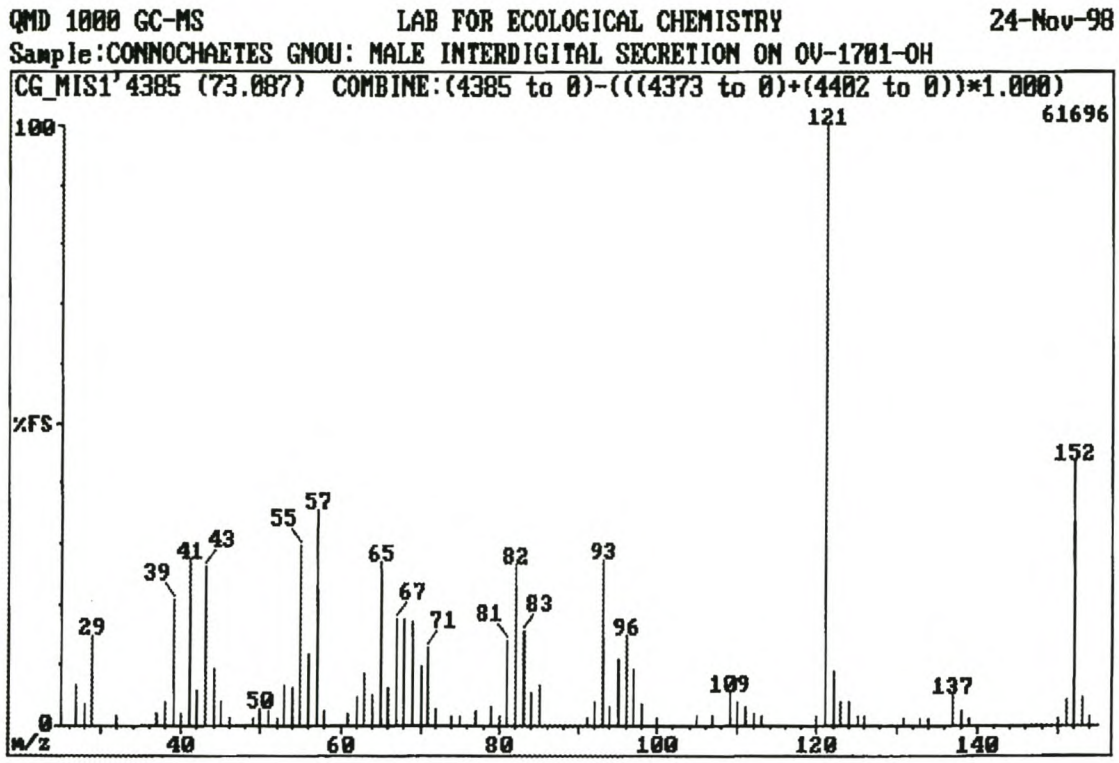


Fig. 2.99: EI mass spectrum of component 4385 (methyl 3-hydroxybenzoate)

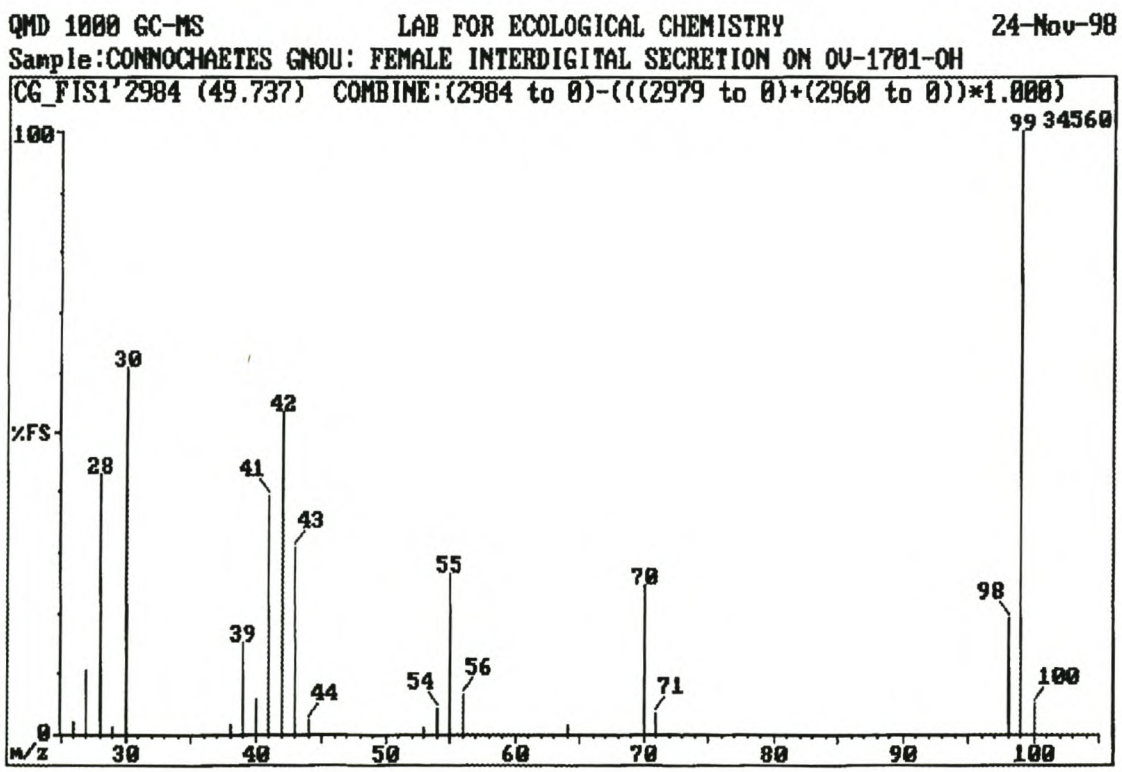


Fig. 2.100: EI mass spectrum of component 2982 (2-piperinone)

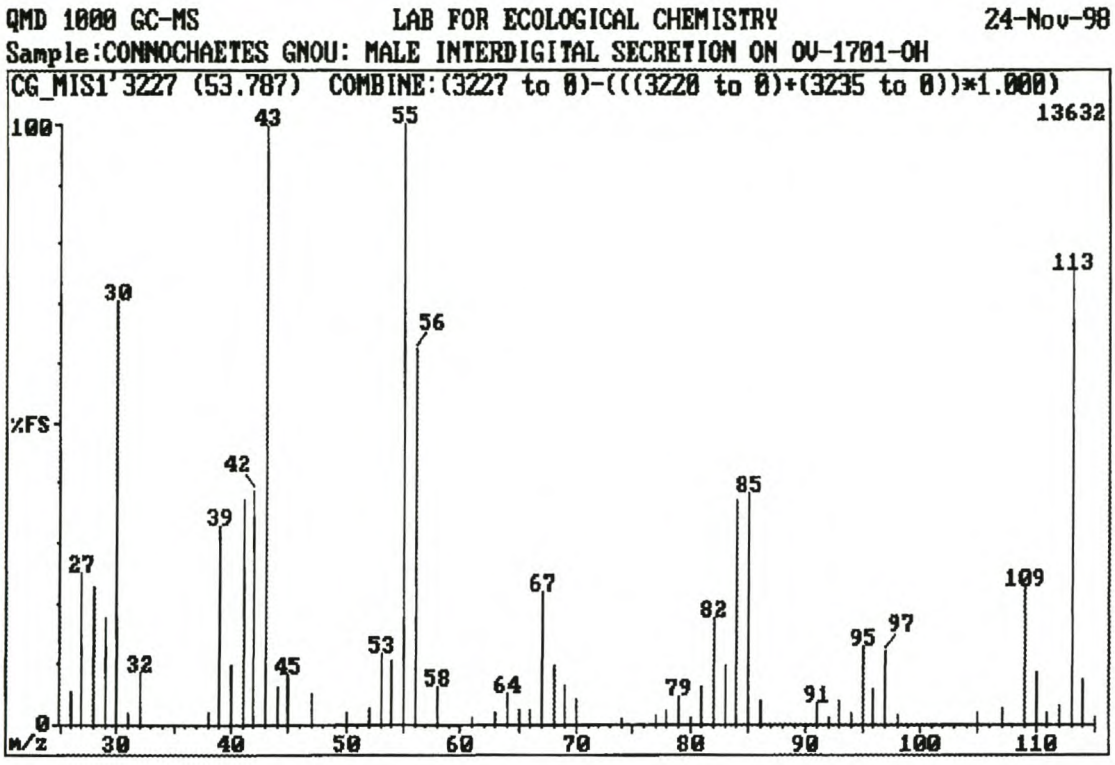


Fig. 2.101: EI mass spectrum of component 3227 (6-hexanelactam)

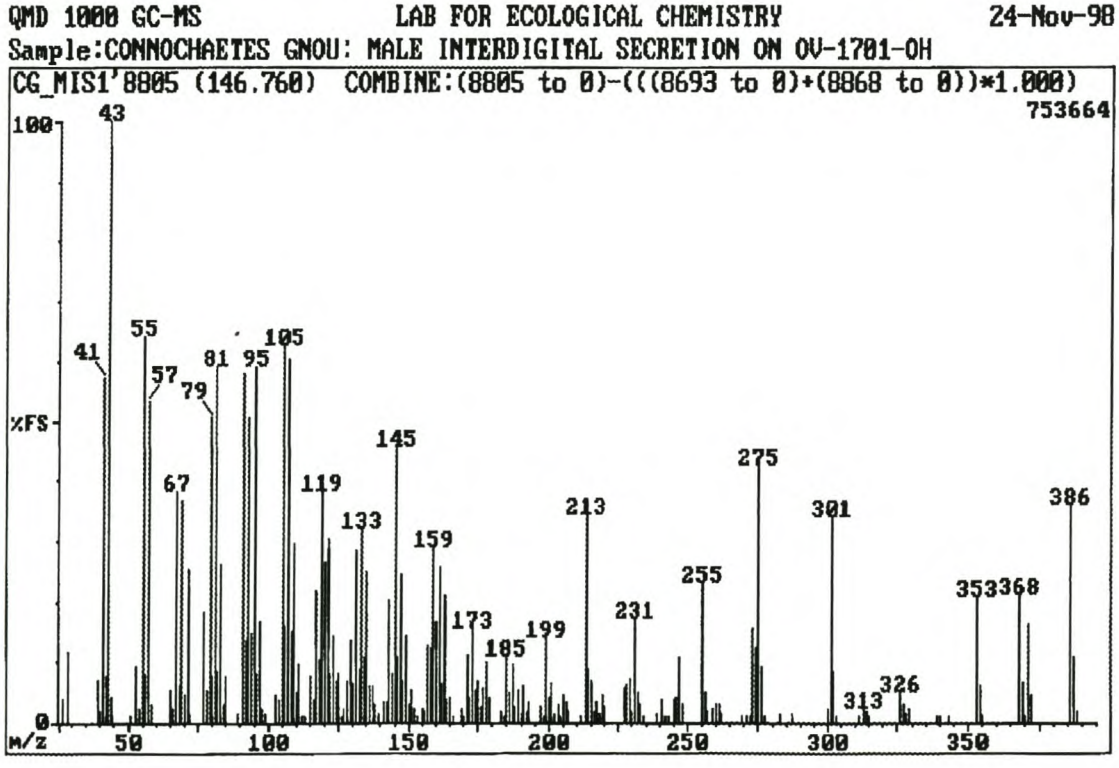


Fig. 2.102: EI mass spectrum of component 8805 (cholesterol)

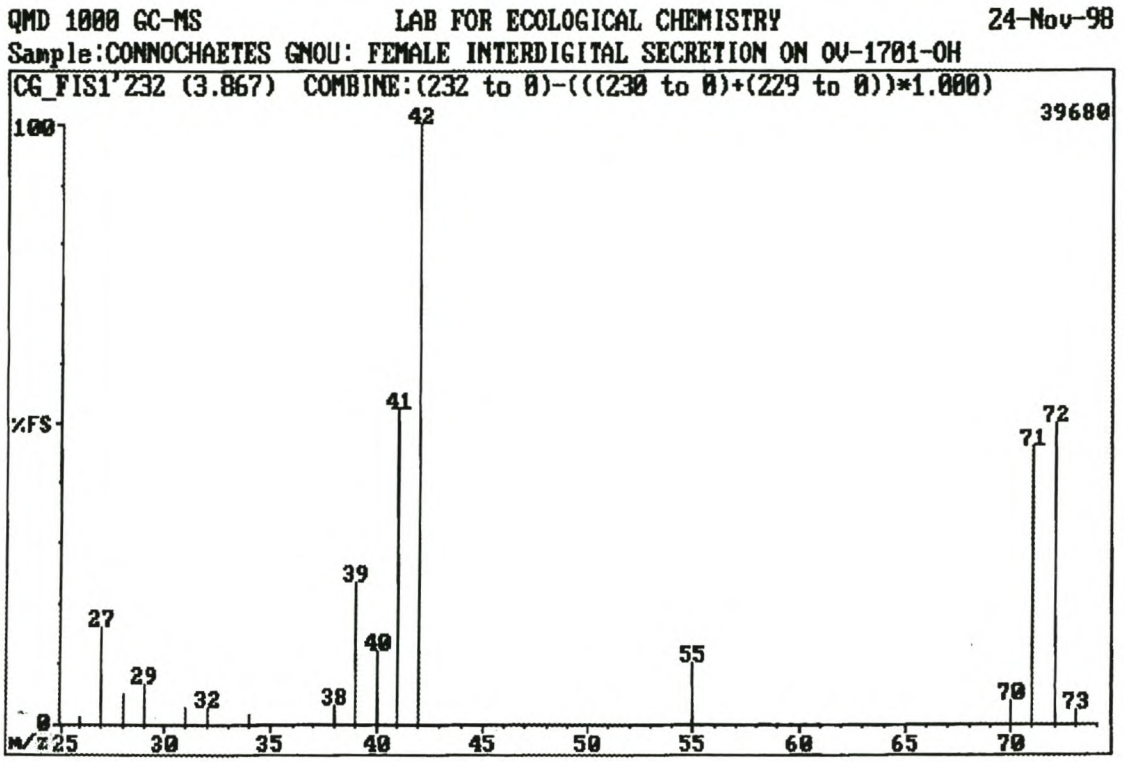


Fig. 2.103: EI mass spectrum of component 232 (tetrahydrofuran)

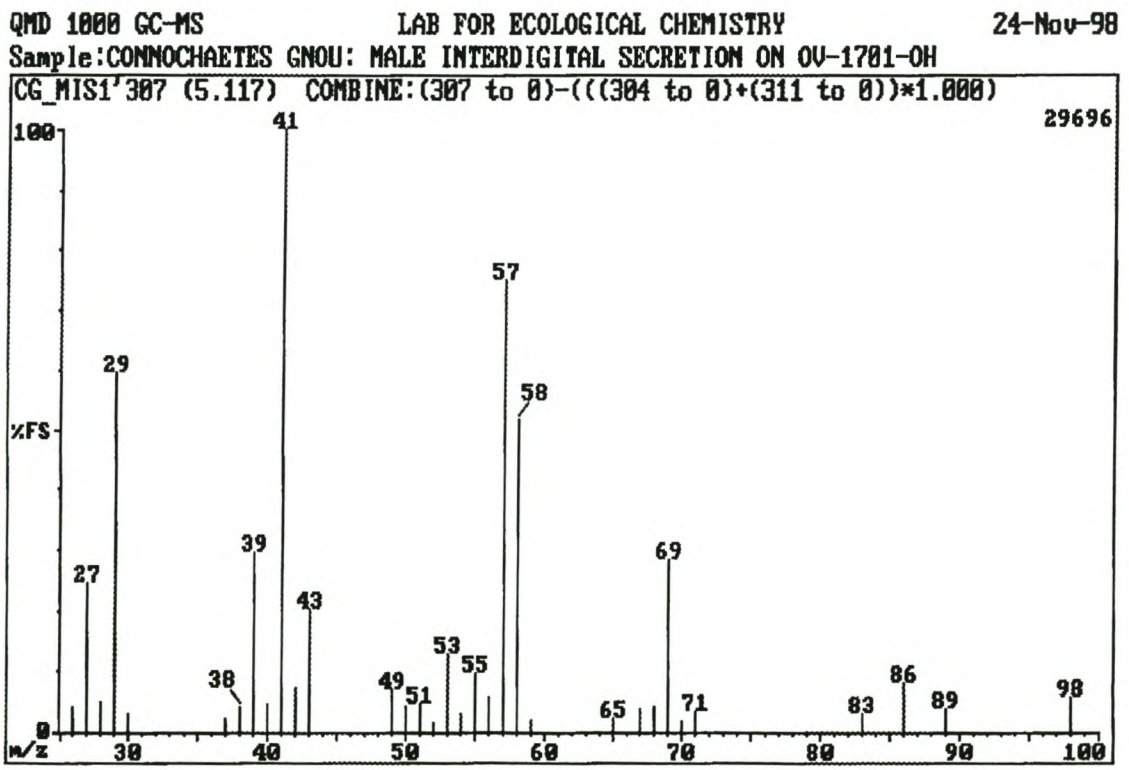


Fig. 2.104: EI mass spectrum of component 307 [(*Z*,*Z*)-dipropenyl ether]

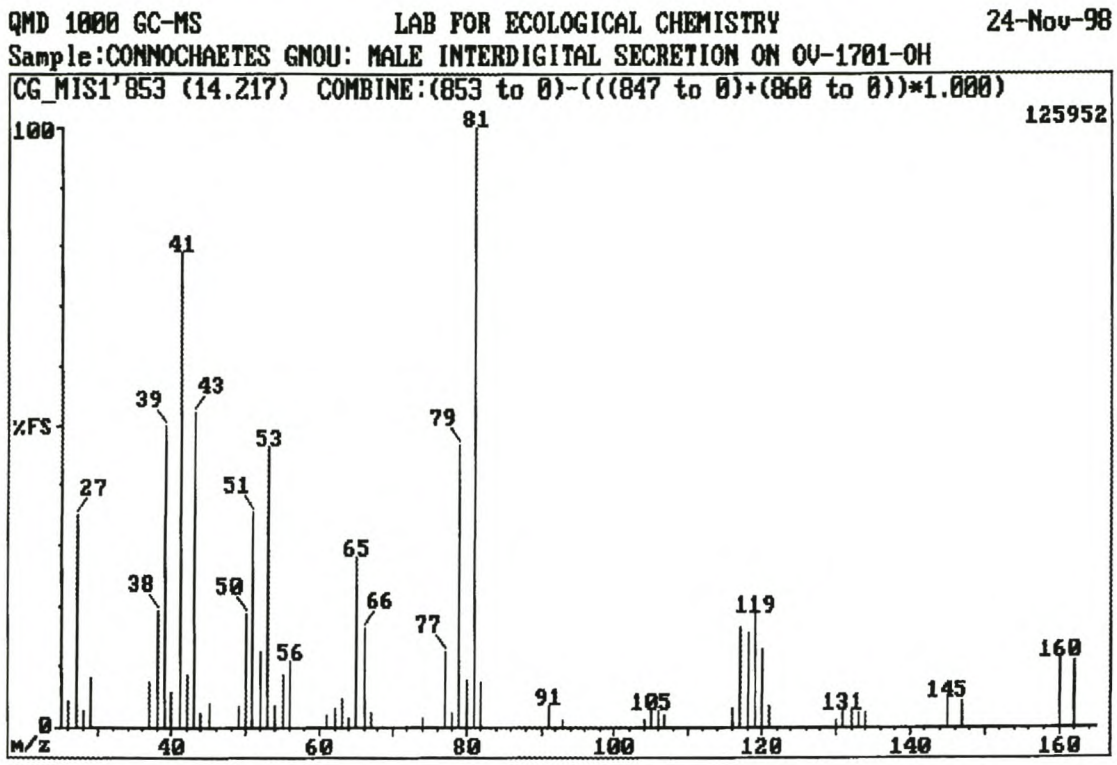


Fig. 2.105: EI mass spectrum of component 853 (1-bromohexyn)

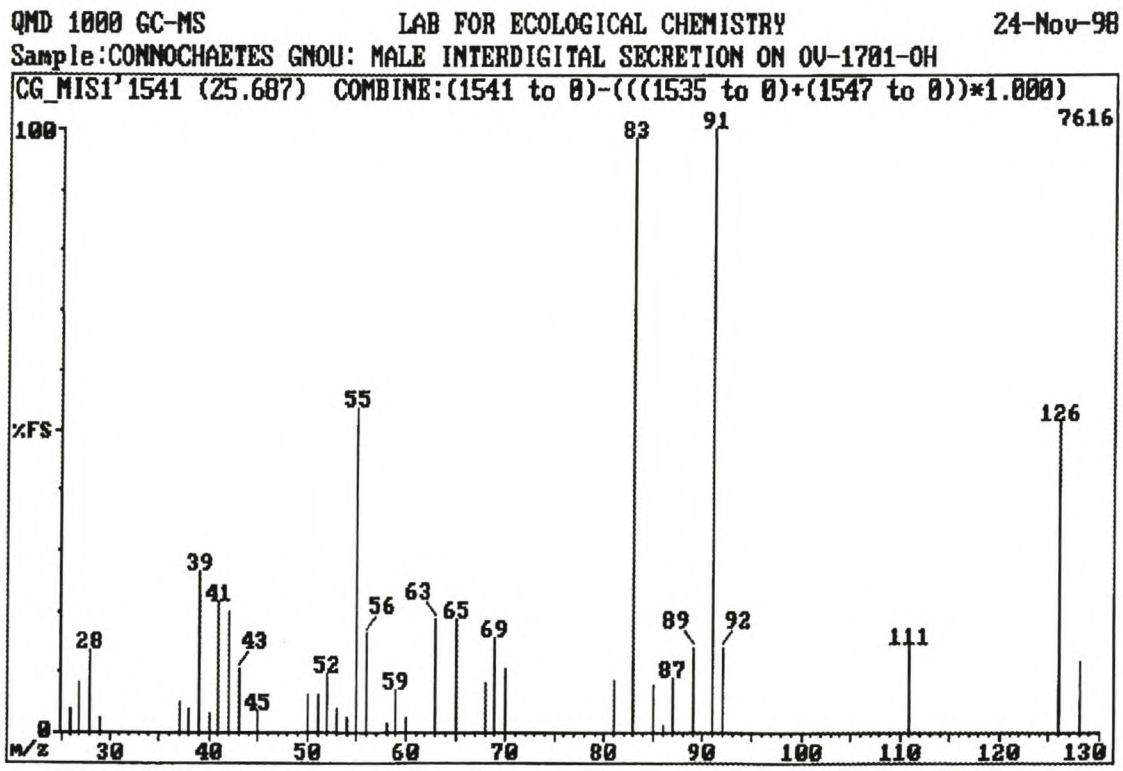


Fig. 2.106 (a): EI mass spectrum of component 1541 (benzyl chloride)

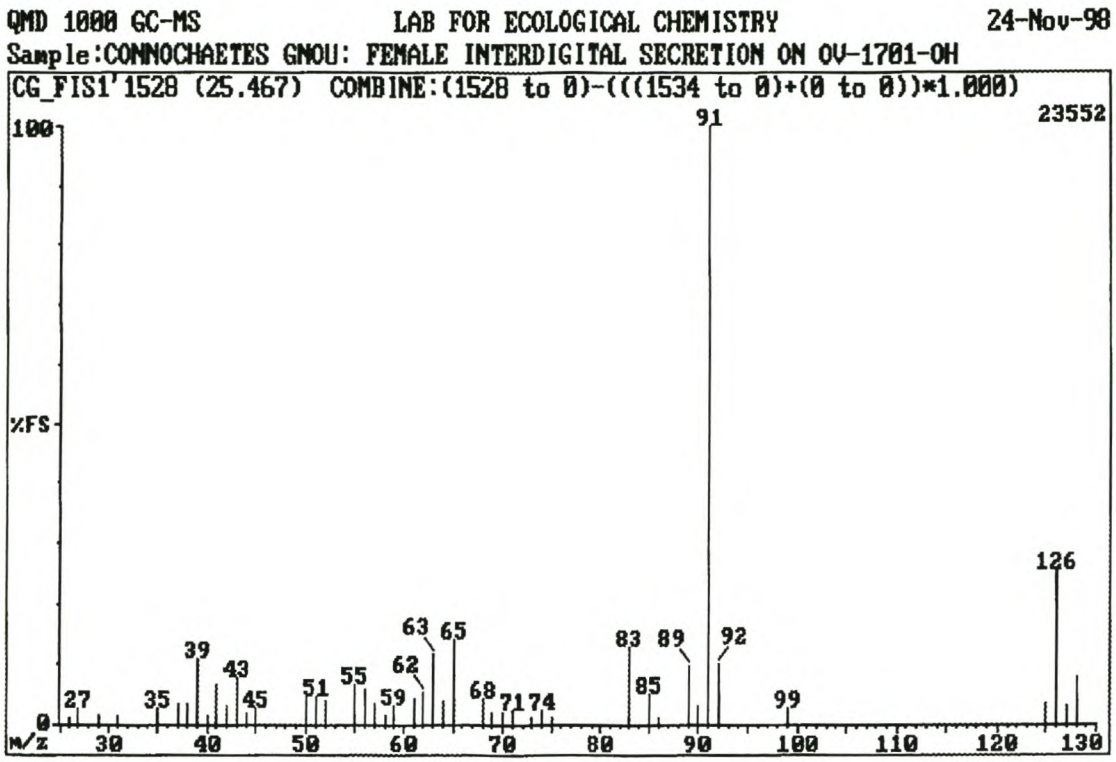


Fig. 2.106 (b): EI mass spectrum of component 1528 (benzyl chloride)

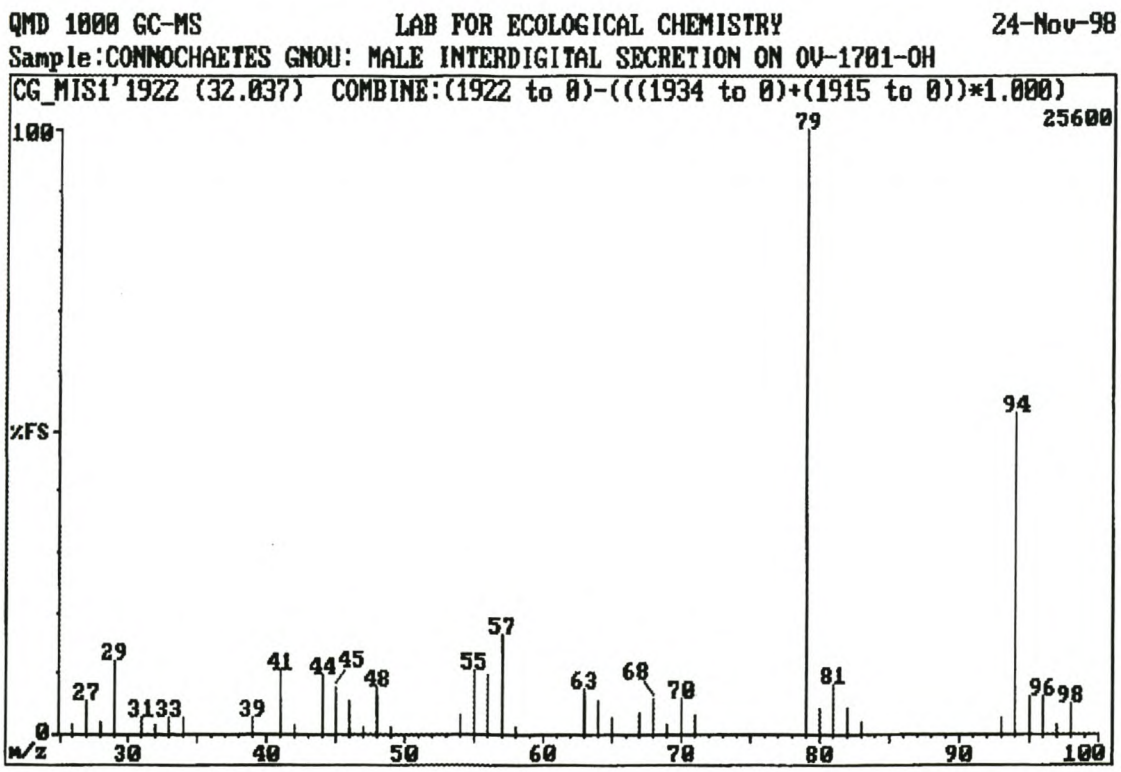


Fig. 2.107: EI mass spectrum of component 1922 (dimethyl sulfone)

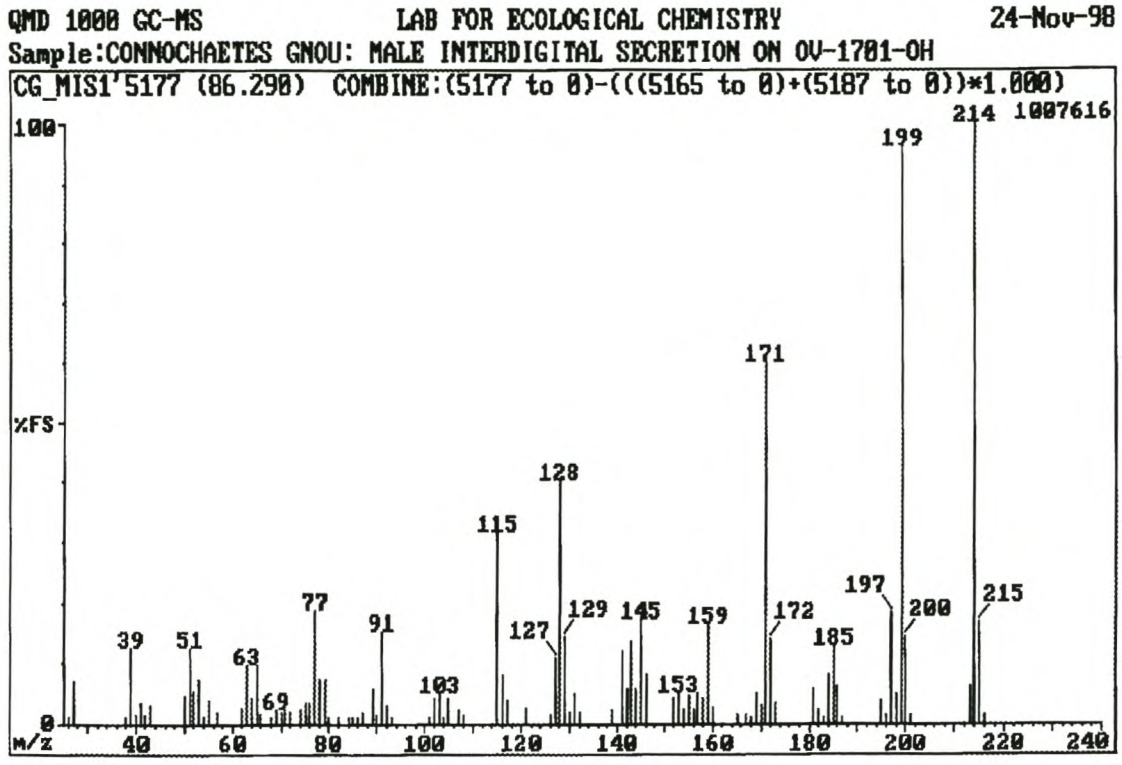


Fig. 2.108: EI mass spectrum of component 5177 (Pummerer's ketone)

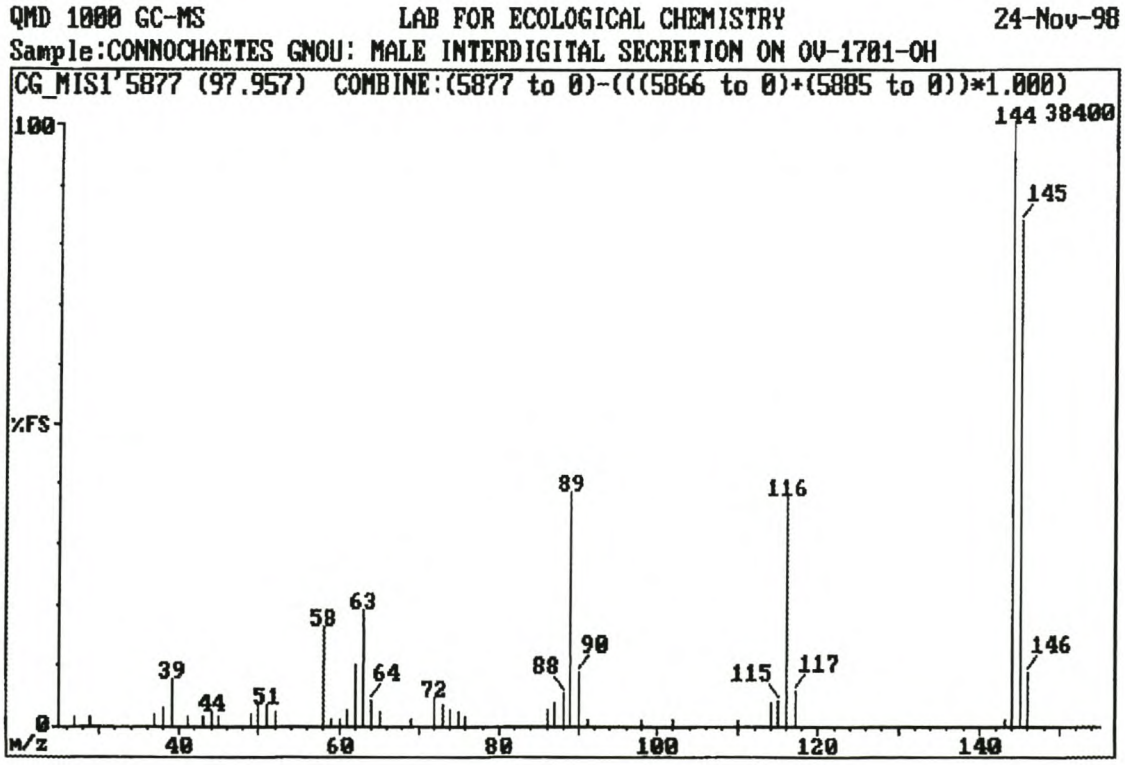


Fig. 2.109: EI mass spectrum of component 5877 (1*H*-indole-3-carboxaldehyde)

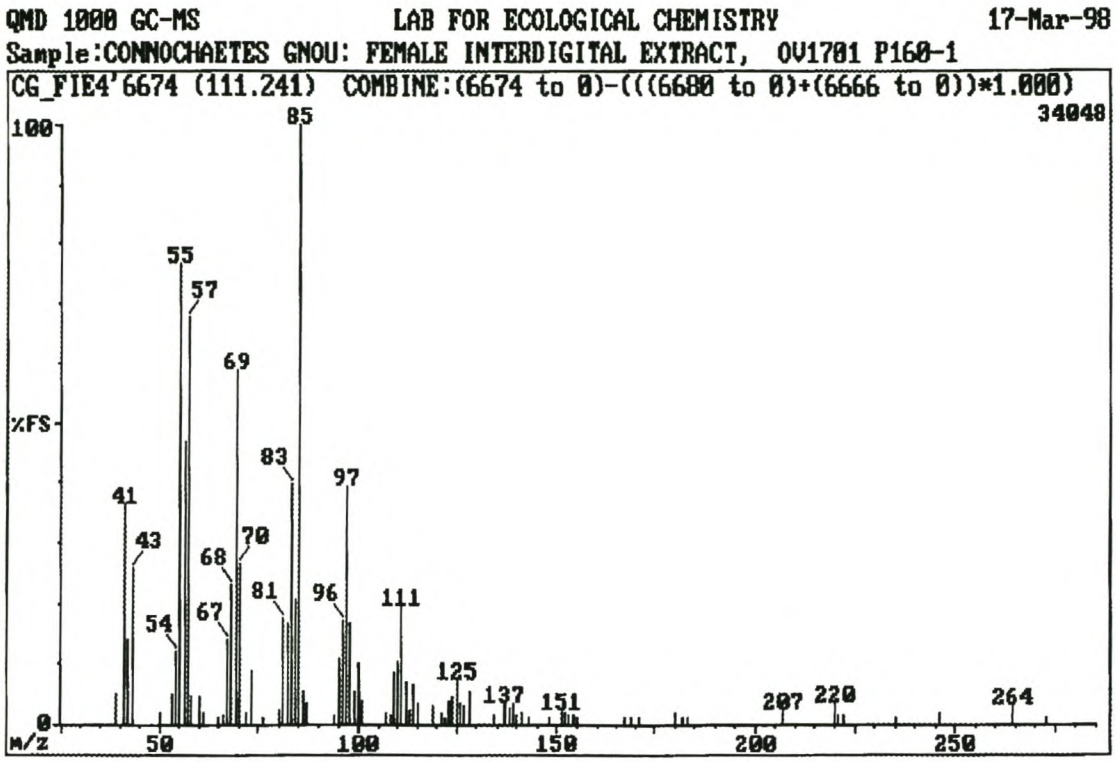


Fig. 2.110: EI mass spectrum of component 6593 (4-octadecanolide)

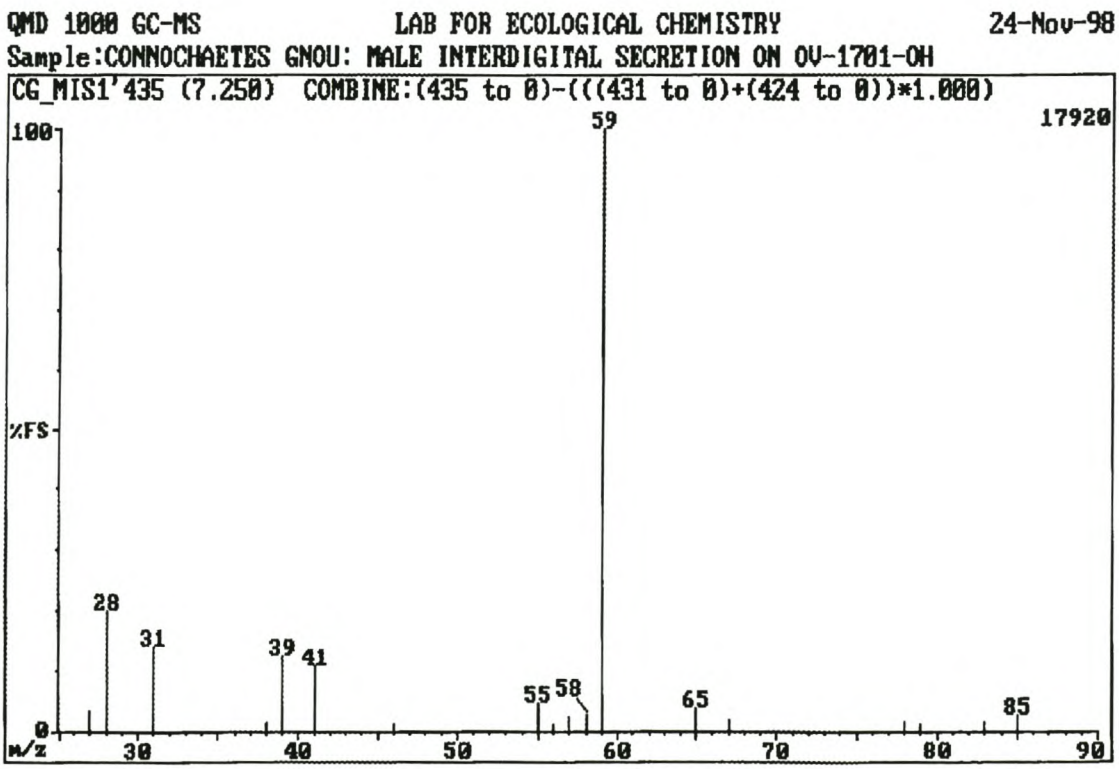


Fig. 2.111: EI mass spectrum of unidentified component 435

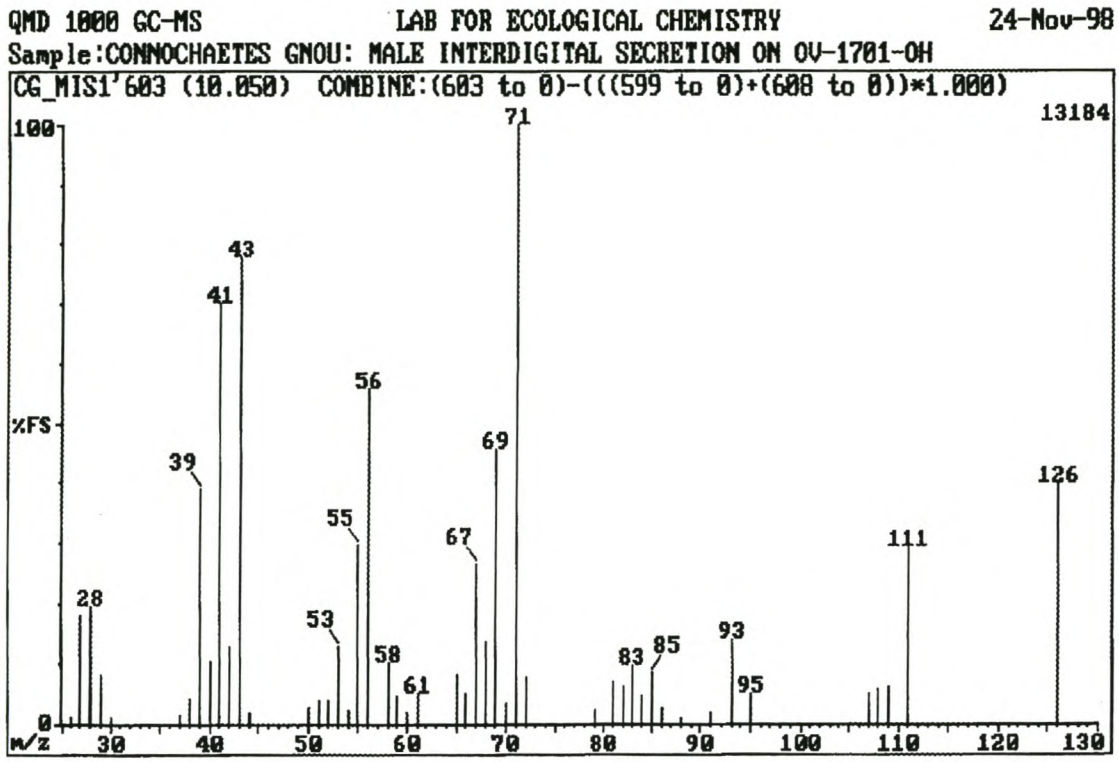


Fig. 2.112: EI mass spectrum of unidentified component 603

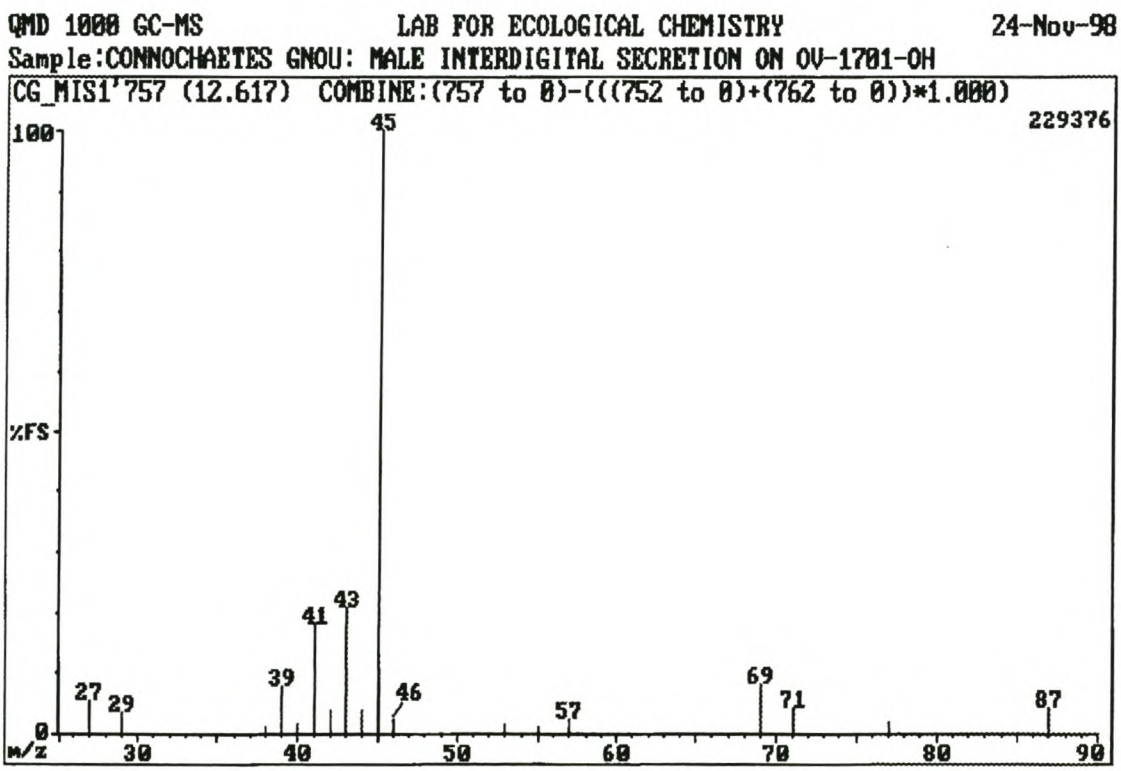


Fig. 2.113: EI mass spectrum of unidentified component 757

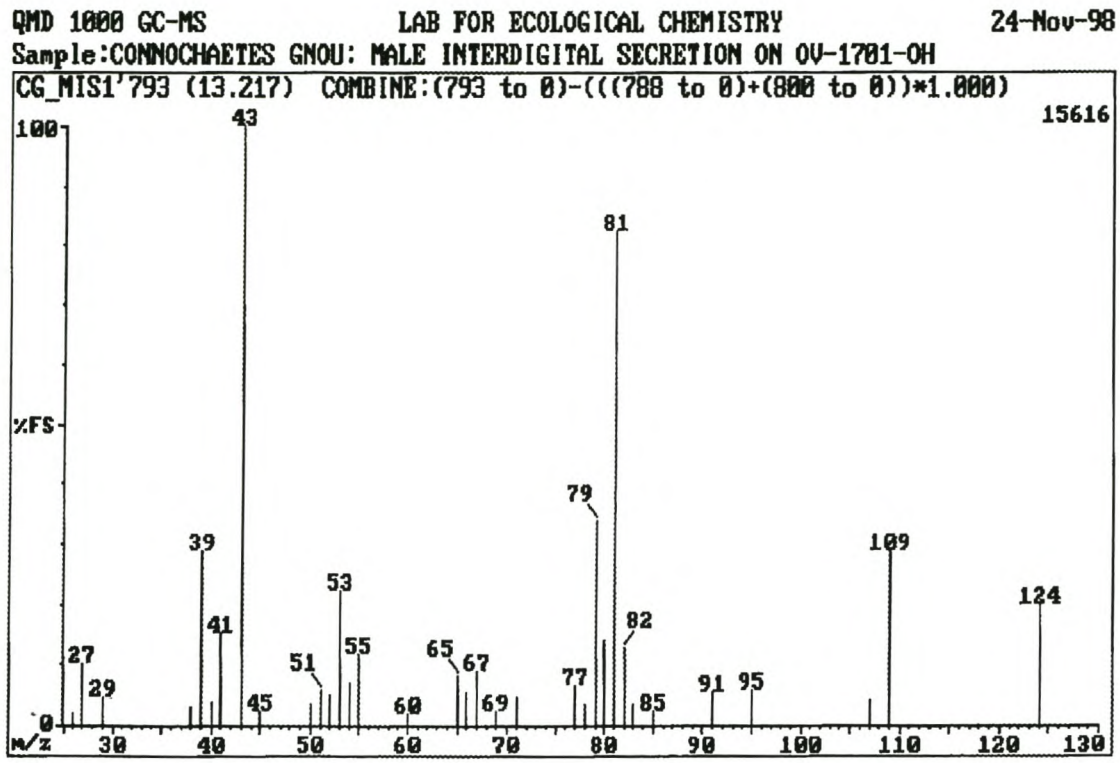


Fig. 2.114: EI mass spectrum of unidentified component 793

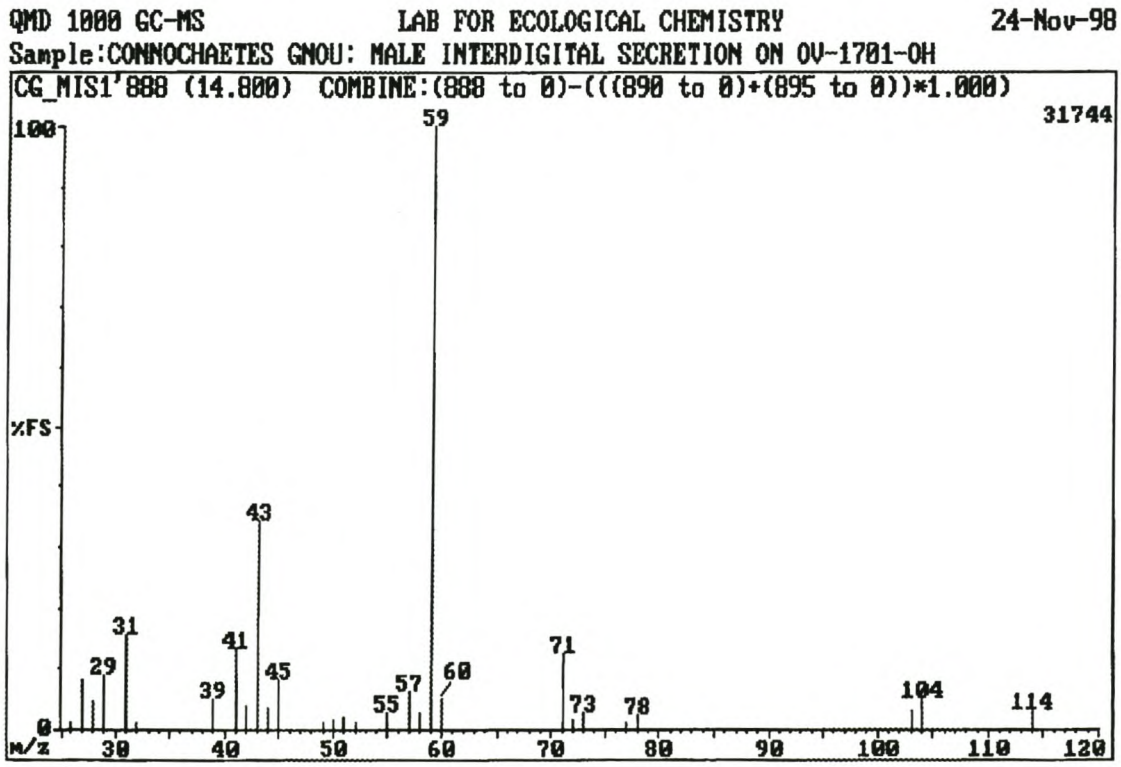


Fig. 2.115: EI mass spectrum of unidentified component 888

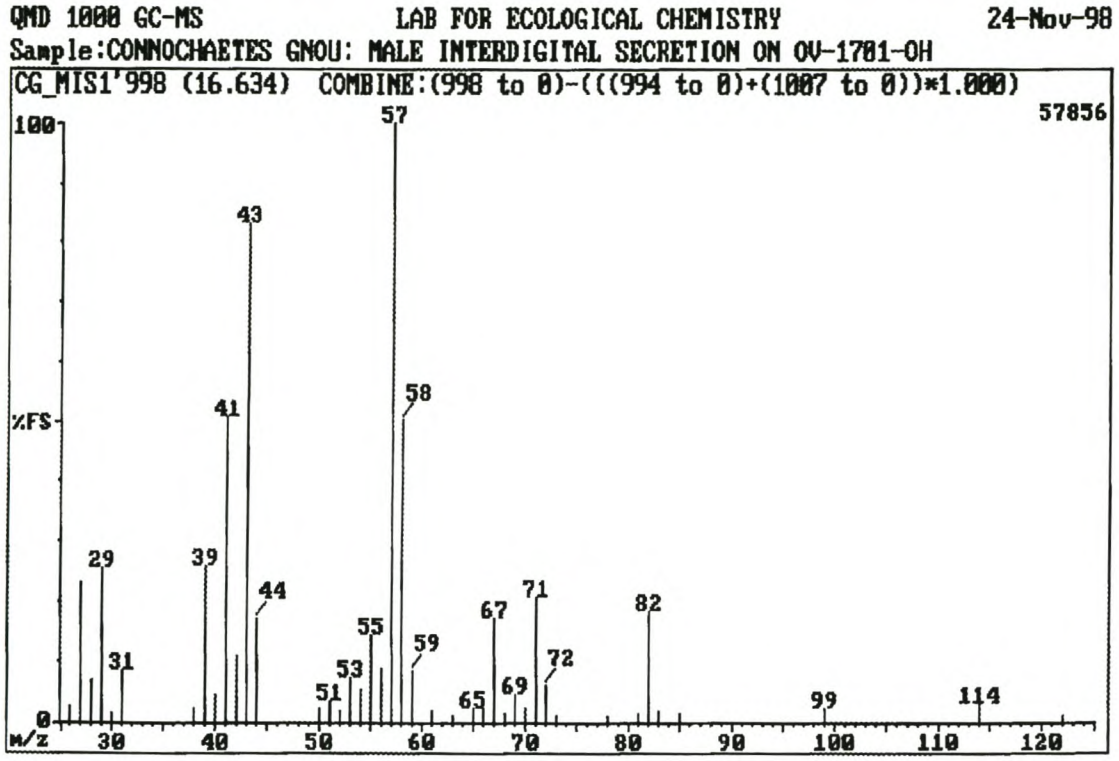


Fig. 2.116: EI mass spectrum of unidentified component 998

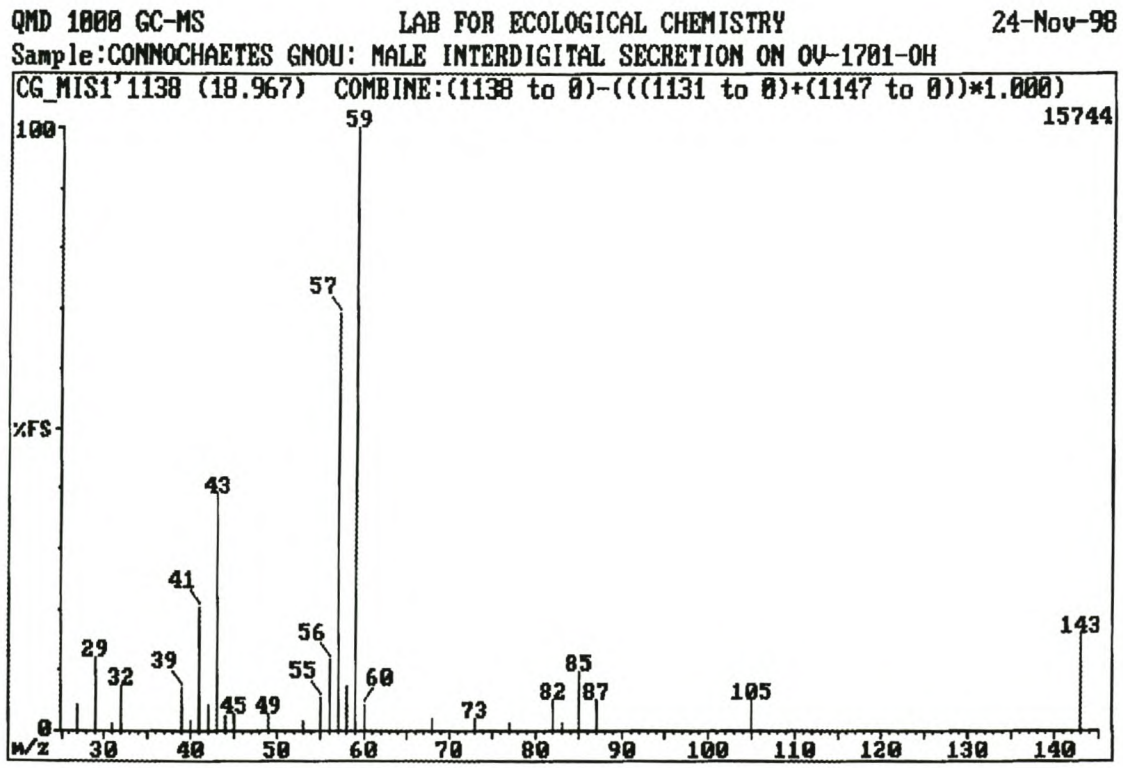


Fig. 2.117: EI mass spectrum of unidentified component 1138

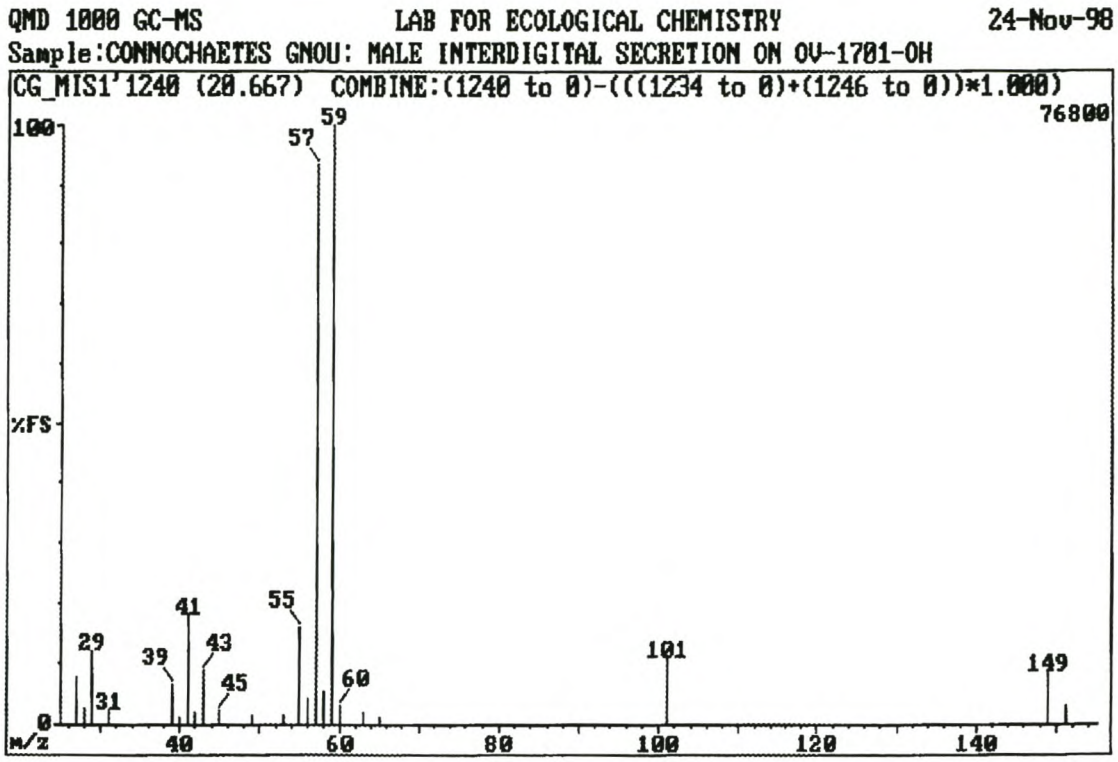


Fig. 2.118: EI mass spectrum of unidentified component 1240

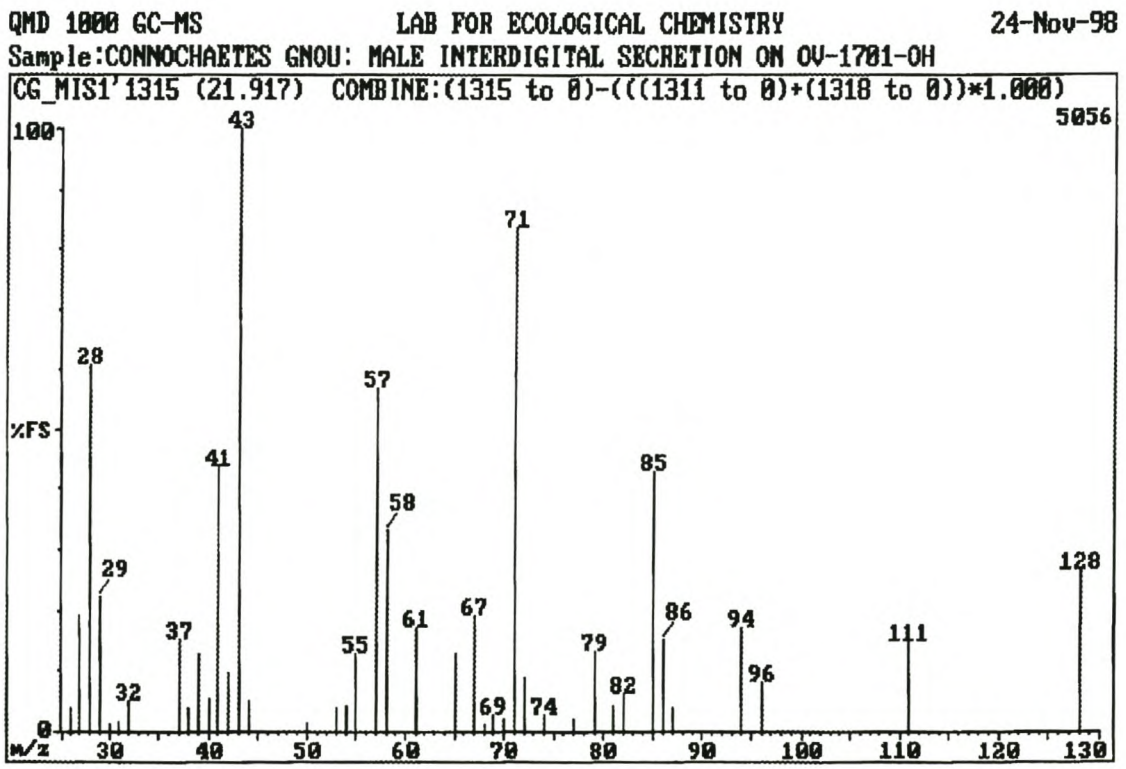


Fig. 2.119: EI mass spectrum of unidentified component 1315

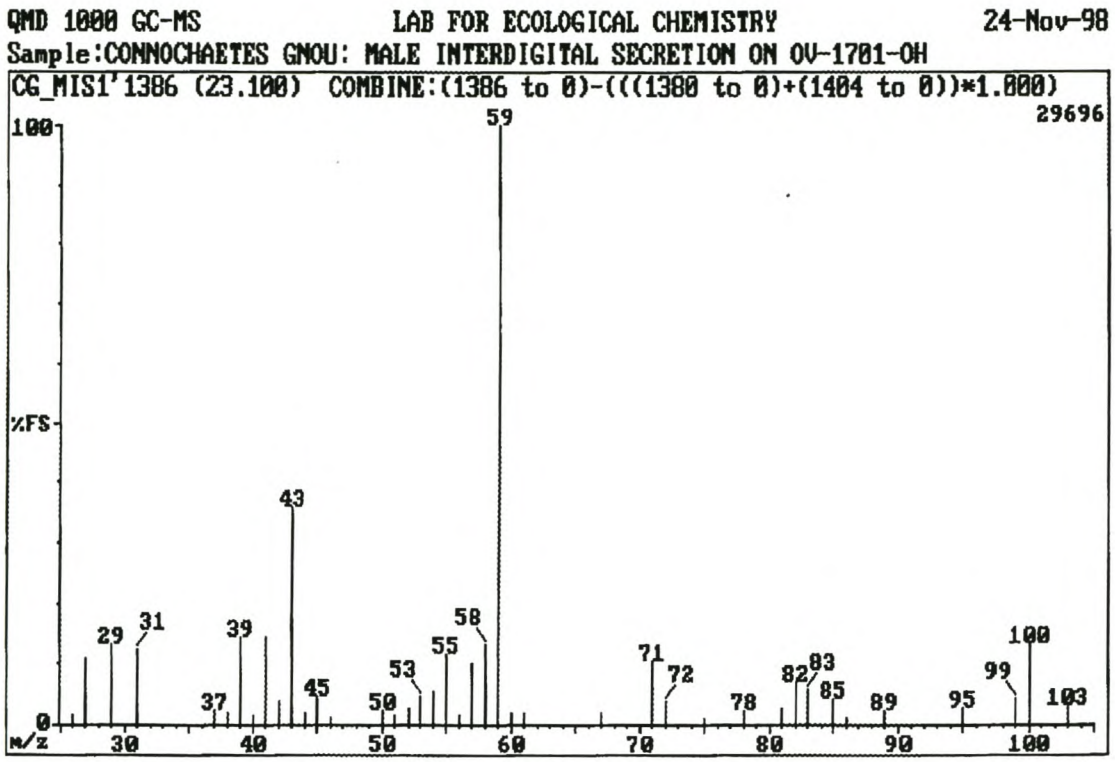


Fig. 2.120: EI mass spectrum of unidentified component 1386

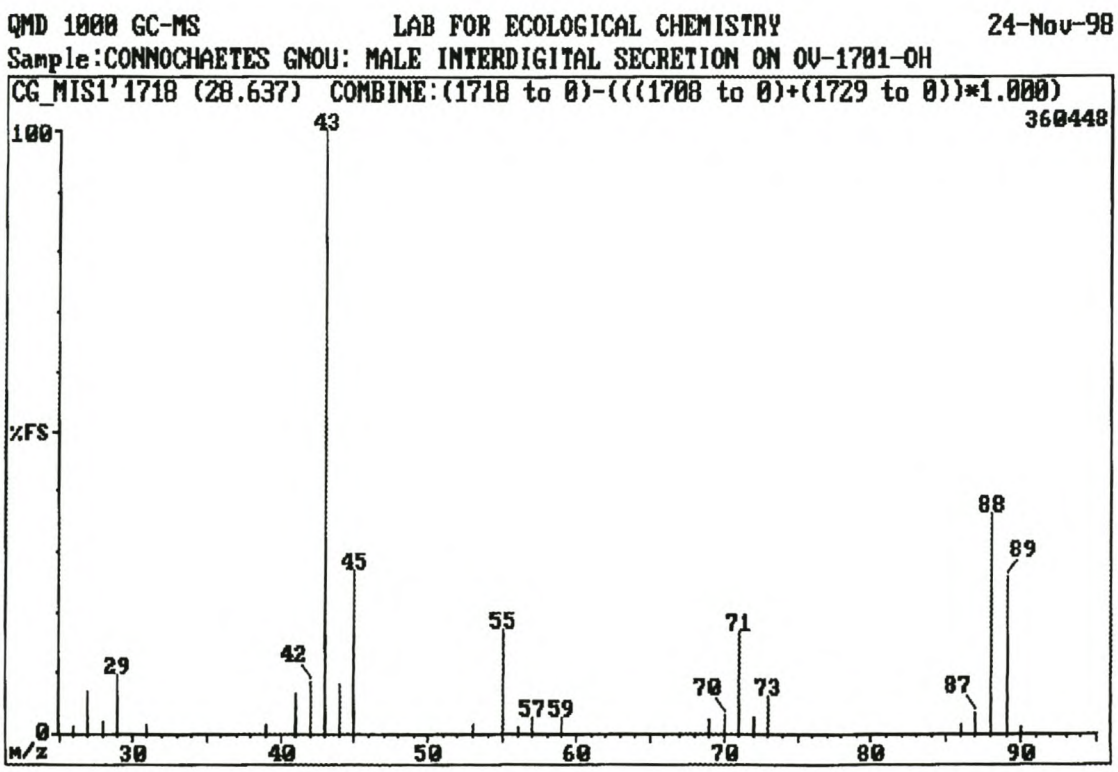


Fig. 2.121: EI mass spectrum of unidentified component 1718

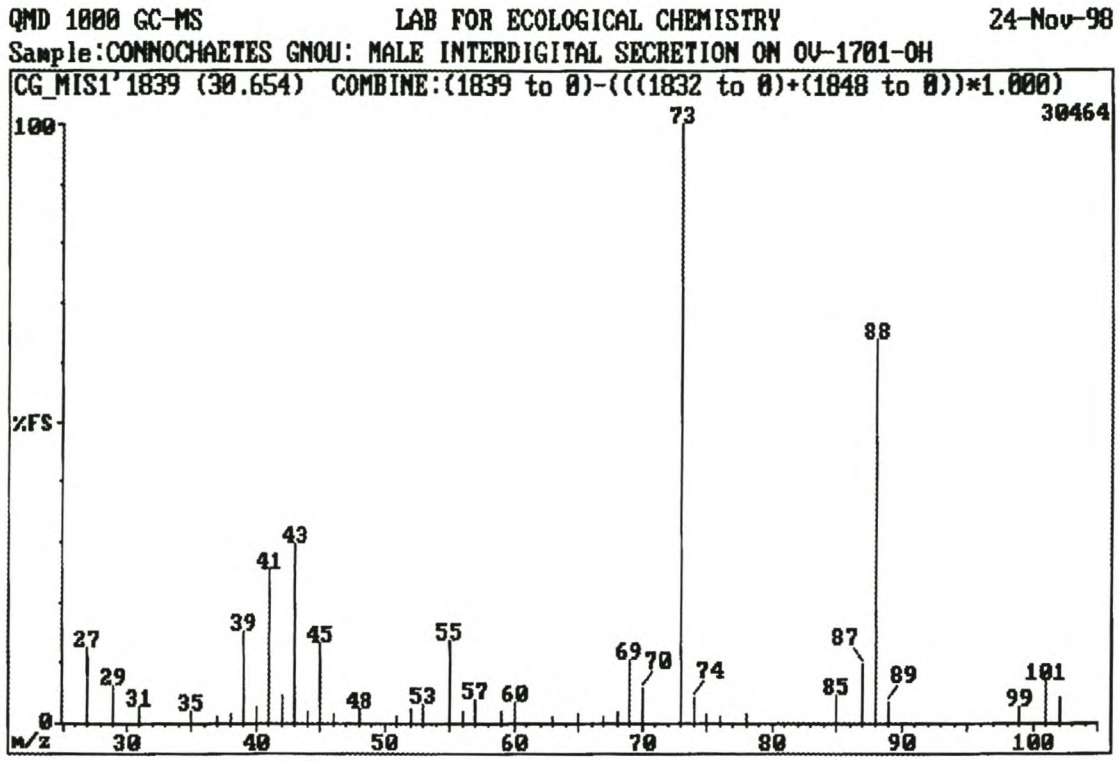


Fig. 2.122: EI mass spectrum of unidentified component 1839

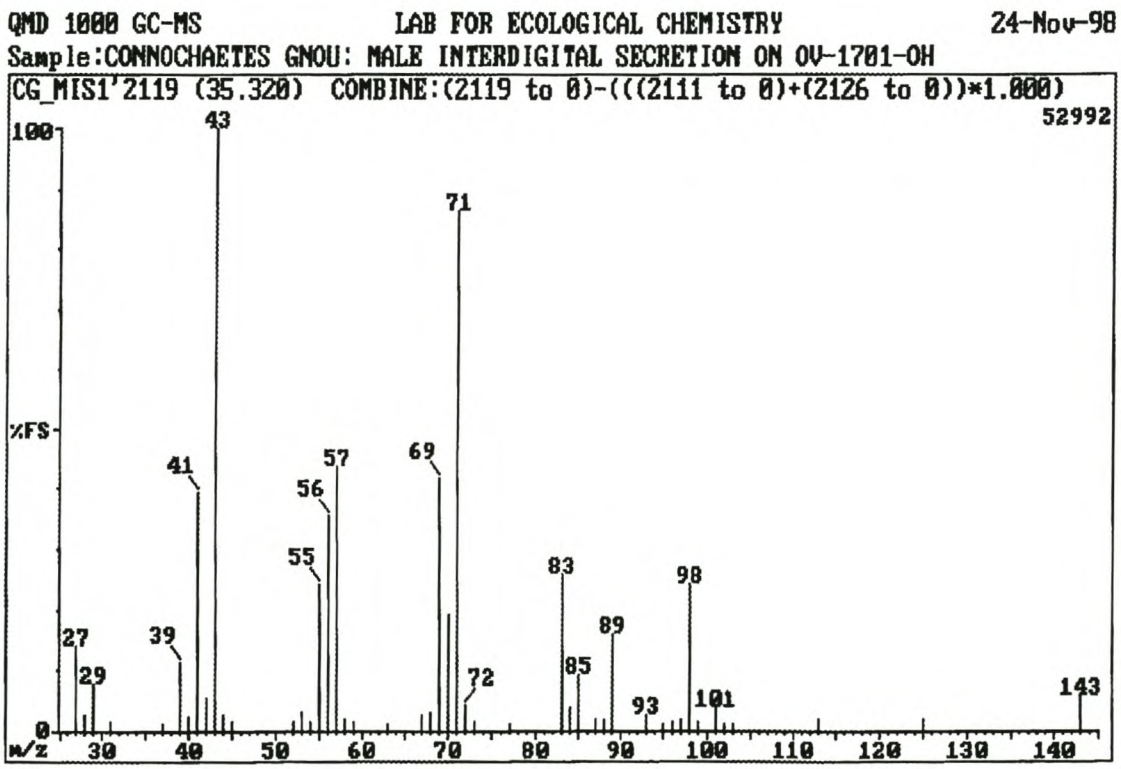


Fig. 2.123: EI mass spectrum of unidentified component 2119

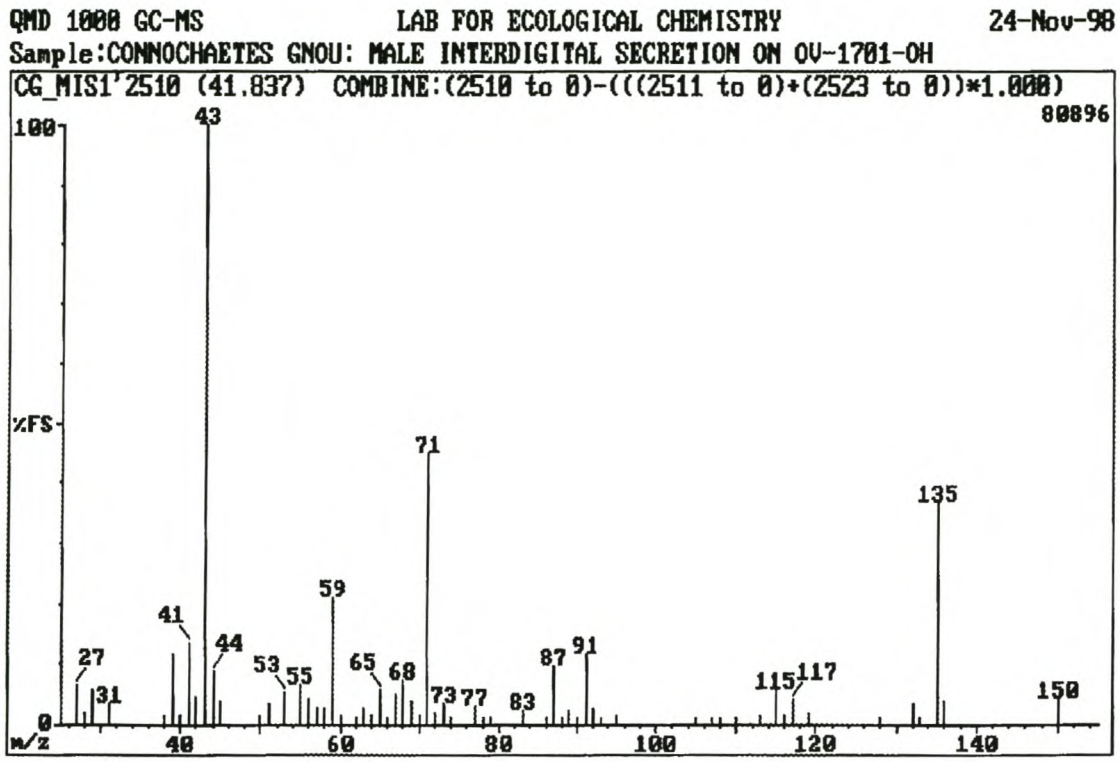


Fig. 2.124: EI mass spectrum of unidentified component 2510

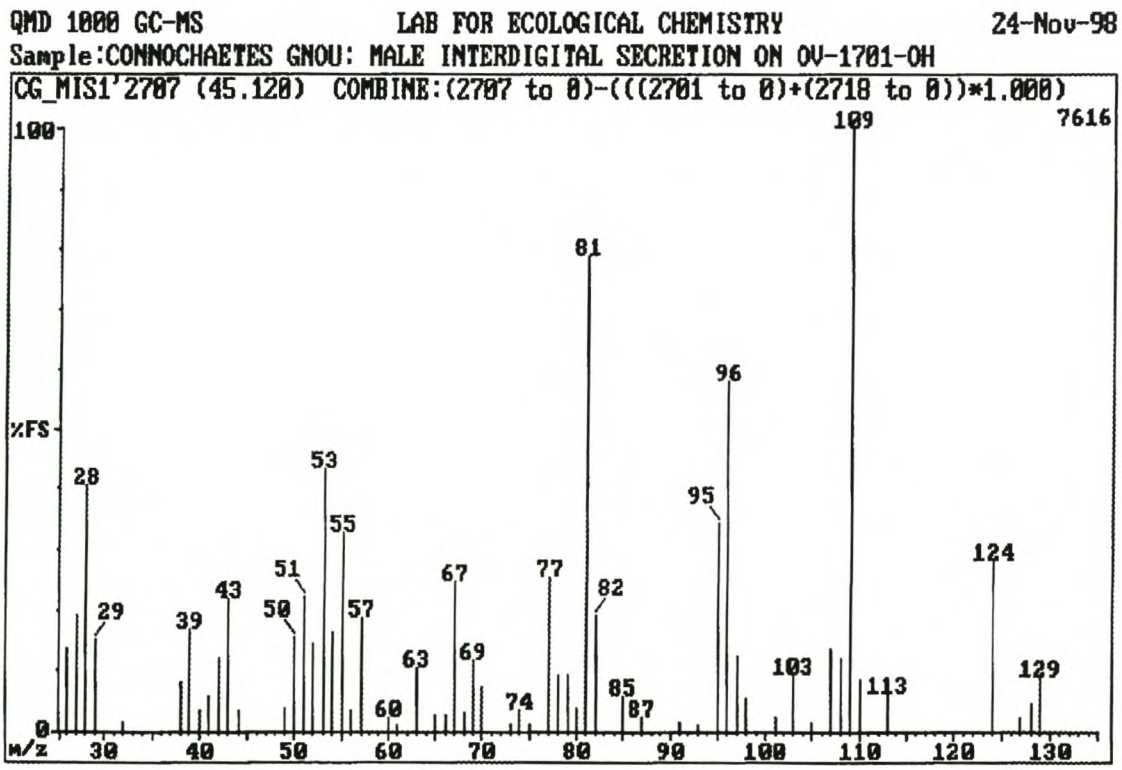


Fig. 2.125: EI mass spectrum of unidentified component 2707

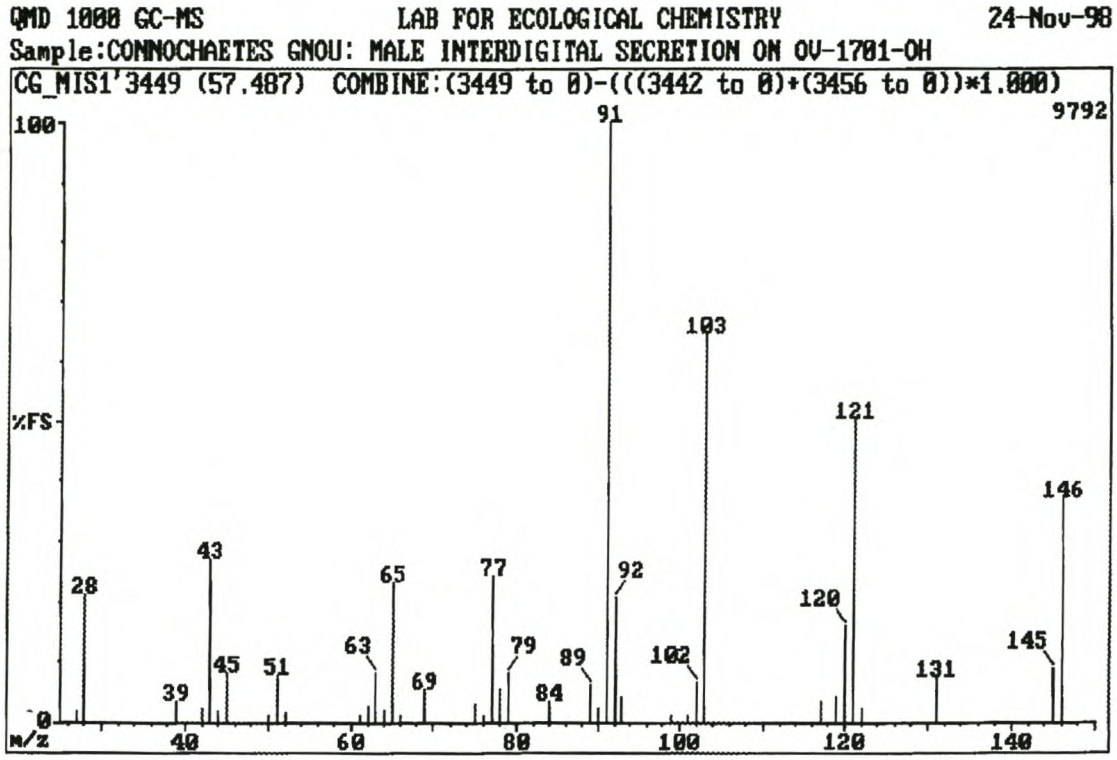


Fig. 2.126: EI mass spectrum of unidentified component 3449

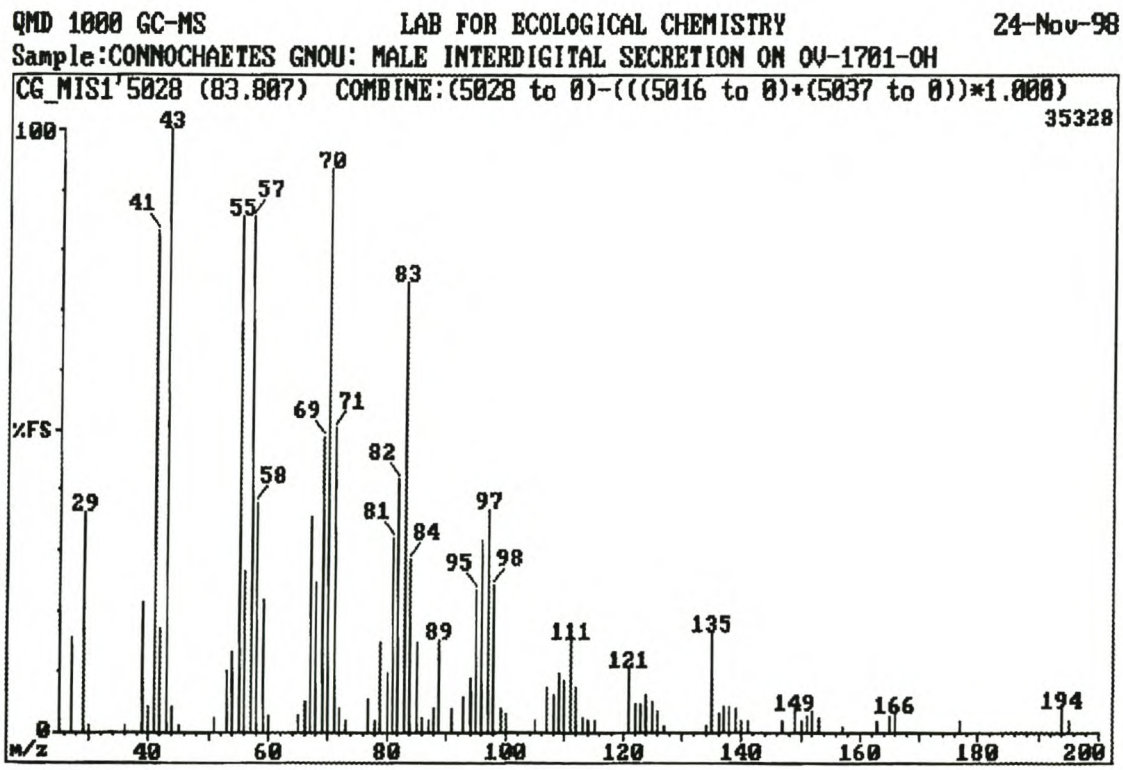


Fig. 2.127: EI mass spectrum of unidentified component 5028

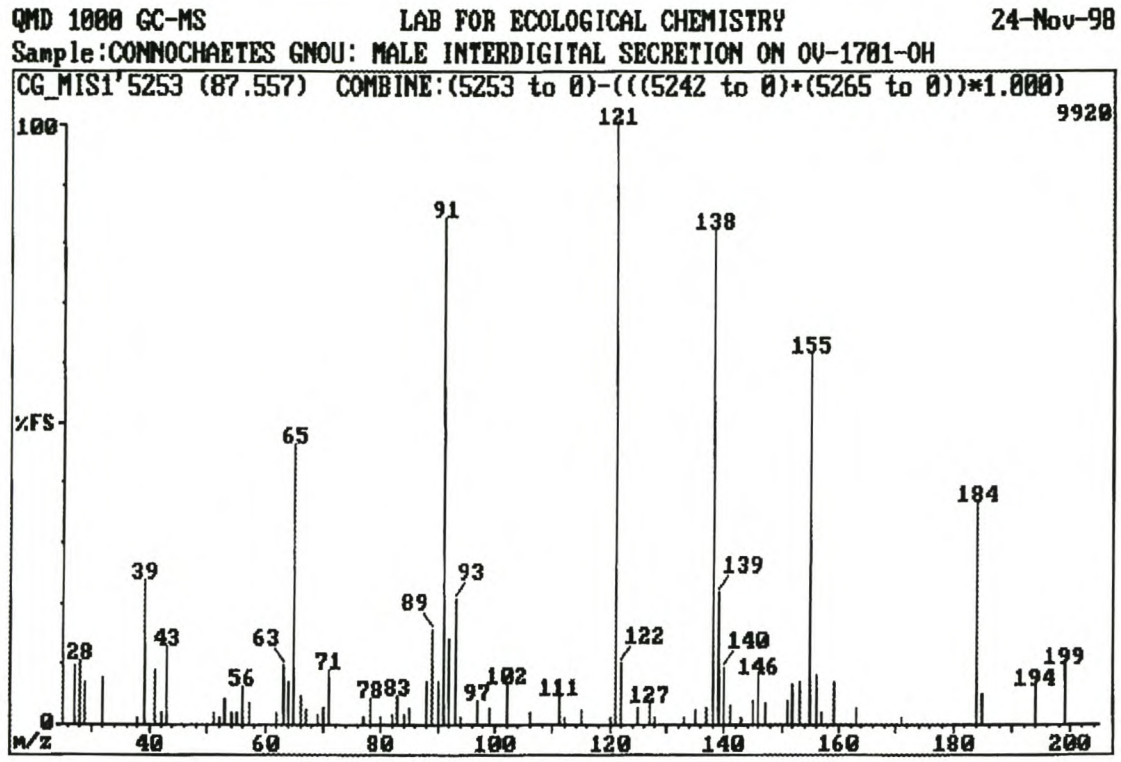


Fig. 2.128: EI mass spectrum of unidentified component 5253

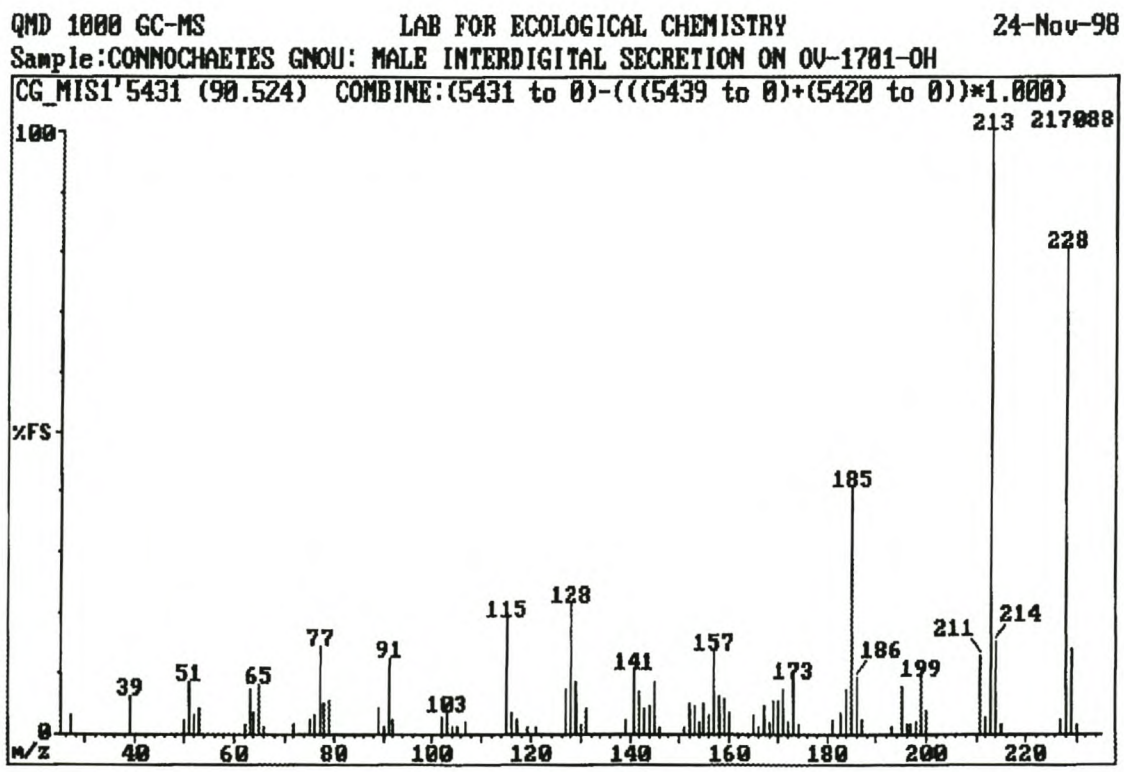


Fig. 2.129: EI mass spectrum of unidentified component 5431

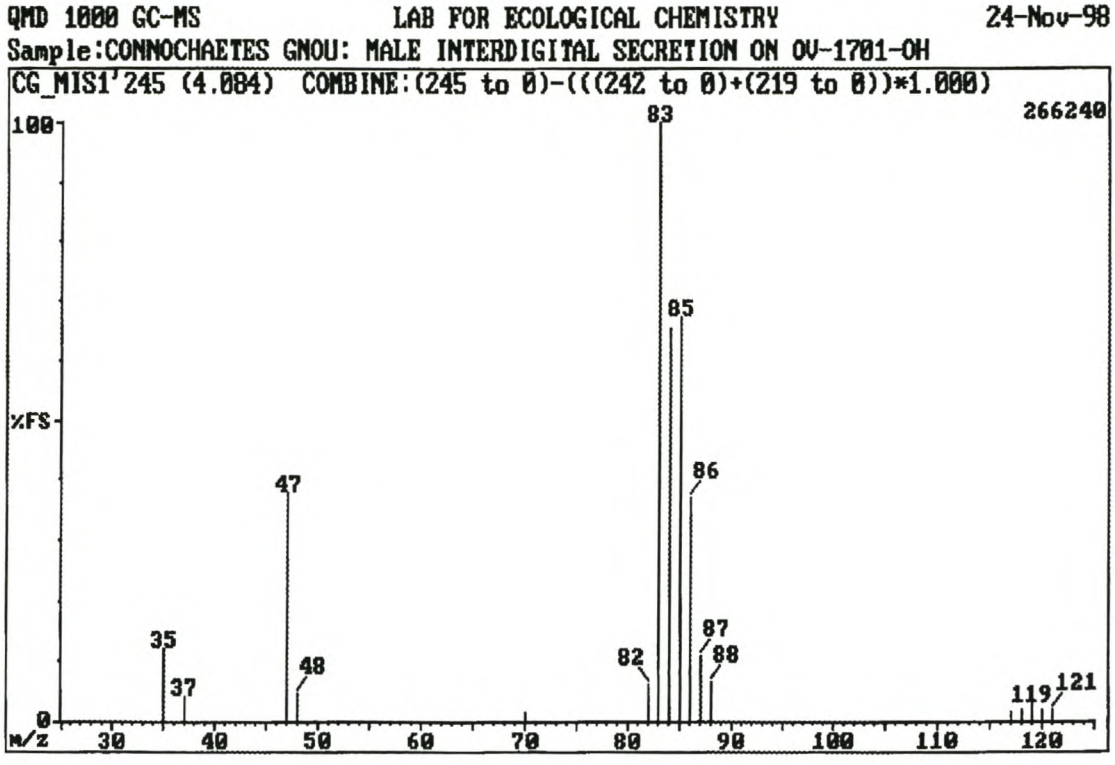


Fig. 2.130: EI mass spectrum of component 245 (chloroform)

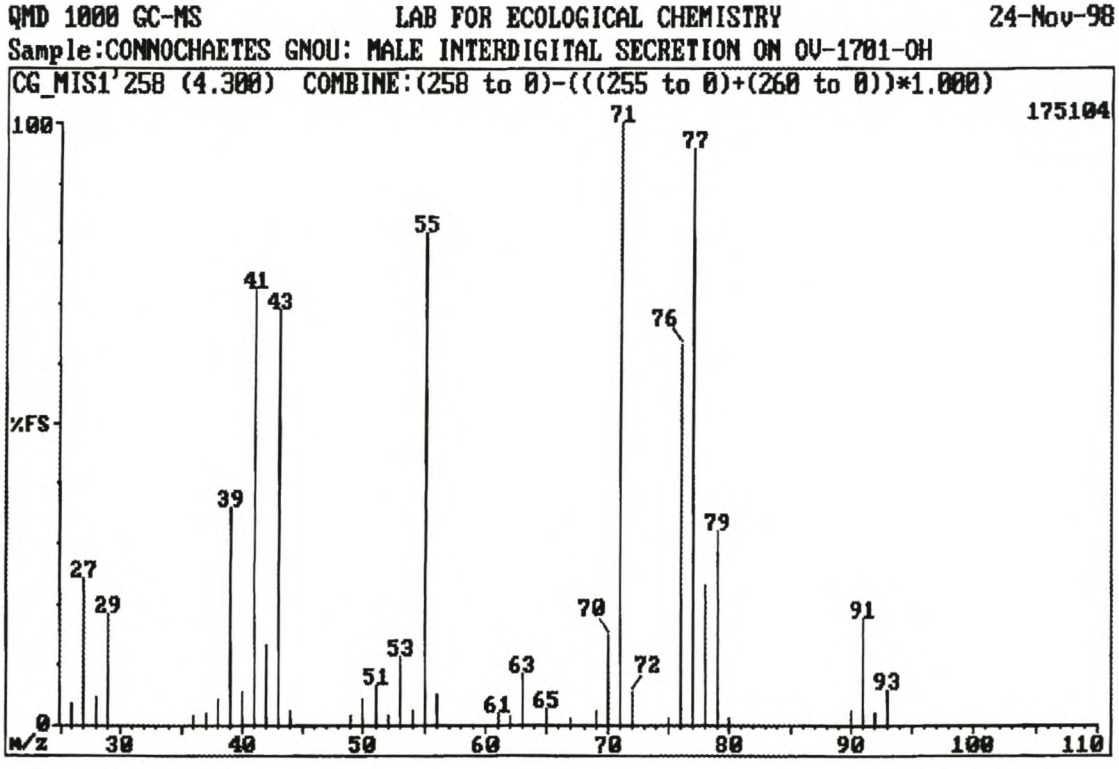


Fig. 2.131: EI mass spectrum of component 258 (2-chloro-2-methylbutane)

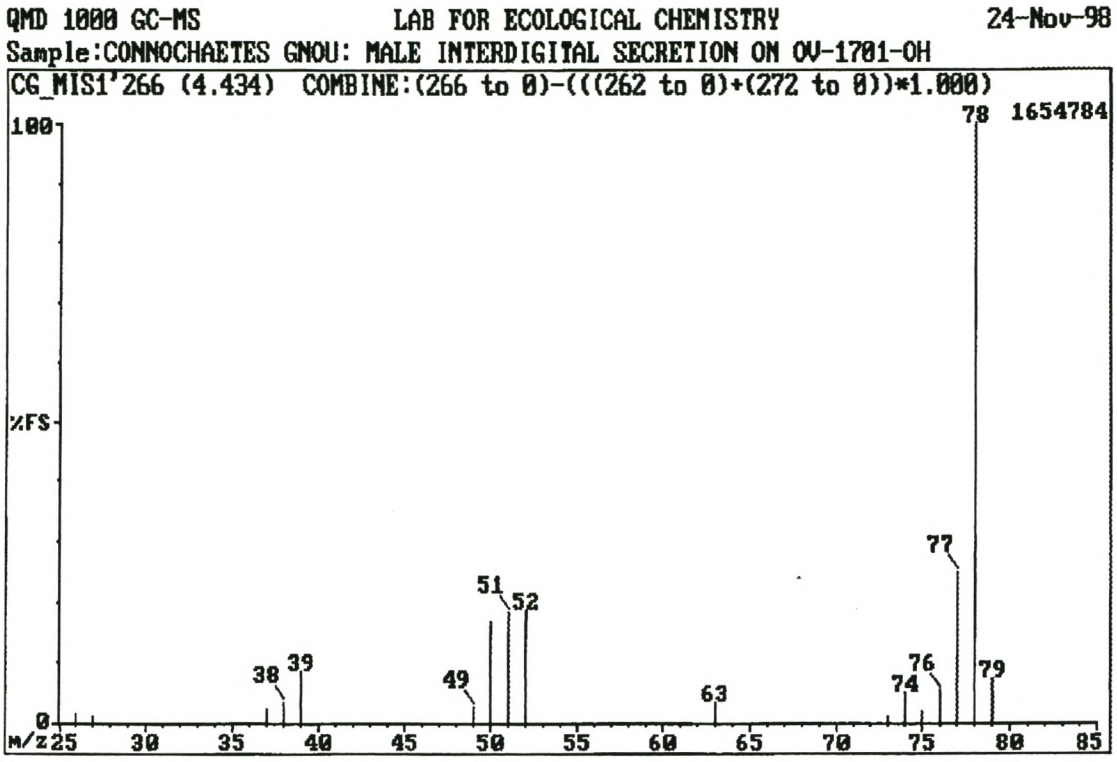


Fig. 2.132: EI mass spectrum of component 266 (benzene)

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CHAPTER 3
EXPERIMENTAL

3.1 General

All Pyrex glassware and the porcelain mortar and pestle used in the preparation and handling of biological material and extracts were heated to 500°C in an annealing oven to remove any traces of organic material. Dichloromethane (Merck, Residue Analysis Grade) was used for extraction purposes. Syringes, stainless-steel needles, *etc.*, were cleaned with this solvent.

3.2 Analytical methods

3.2.1 Gas chromatographic analyses

Gas chromatographic (GC) analyses were carried out with a Carlo Erba 5300 gas chromatograph equipped with a flame ionization detector, Grob split-splitless injector, and a glass capillary column (40 m x 0.3 mm) coated with OV-1701-OH at a film thickness of 0.375 μm. This column was manufactured by the Laboratory for Ecological Chemistry, University of Stellenbosch. All analyses were done with hydrogen as carrier gas at a linear velocity of 50.0 cm/s (column temperature 40°C). The flame ionization detector was operated at 280°C and the injector at 220°C. Samples were injected in the split mode, the analytes thermally focussed on the column at *ca.* 27°C, and analyzed using a temperature programme of 2°C/min from 40 to 285°C (hold).

3.2.2 Gas chromatographic-mass spectrometric analyses

Electron impact (EI) mass spectra were recorded at 70 eV on a Carlo Erba QMD 1000 gas chromatograph-mass spectrometer (GC-MS system) with VG Analytical Lab-Base software, using the columns and conditions described above, except that helium was used as carrier gas at a linear velocity of 28.6 cm/s (column temperature 40°C). An interface temperature of 250°C was used. The ion

source temperature was set at 180°C and the pressure in the source housing was *ca.* 2 x 10^{-5} mm Hg at a column temperature of 40°C, decreasing to *ca.* 1 x 10^{-5} mm Hg towards the end of the temperature programme. A scan rate of 0.9 scan/sec, with interval of 0.1 seconds between scans, was employed.

3.3 Sample collection and preparation

Interdigital secretions were collected from black wildebeest captured in the Oviston Nature Reserve on the Orange River (Eastern Cape). Samples were taken from both sexes. Surgical gauze squares (*ca.* 25 mm x 25 mm) consisting of several layers of surgical gauze were extracted for five hours with dichloromethane (Residue Analysis Grade), dried in an atmosphere of purified N_2 (activated charcoal) and stored in glass-stoppered bottles. Interdigital secretion was collected by rolling a gauze square around the tip of dressing forceps, inserting the forceps with gauze into the interdigital cavity and collecting the secretion by rotating the forceps while removing it from the cavity. The gauze pads with the yellowish secretion were stored at -30°C in glass bottles with Teflon-lined screw caps until used for analysis. Initially the secretion was extracted from the gauze with a minimum of dichloromethane in the smallest possible Soxhlet extractor. The problem with this method is that the extract has to be concentrated for further work by evaporation of a considerable volume of dichloromethane, possibly resulting in the loss of some of the more volatile constituents of the secretion. The solvent was evaporated by placing the vial containing the extract in a 2 litre glass beaker covered with aluminium foil and the solvent vapor purged from the beaker with purified N_2 (activated charcoal) without blowing the purge gas directly into the vial containing the extract. Depending on the concentration of the extract and the size of the vial, the removal of 5 ml of dichloromethane took up to 10 hours. To avoid the use of large volumes of solvent, the following method was also used. A glass vial containing dichloromethane (5 ml or less) and the gauze pads with which the secretions of two animals had been collected, was centrifuged for 1 minute at 1500 r.p.m. to improve contact between the gauze and the small volume of solvent by compressing the gauze in the vial. The material in the vial was sonicated in an ultrasonic bath for 2 minutes, whereafter the extract was separated from the gauze by centrifuging the gauze and solvent in a

sintered glass filter insert suspended in a 5 ml Reacti-Vial (Pierce Chem. Co.). The extract was concentrated as described above. A comparison of this extract and an extract obtained by subsequent extraction of any residual material from the gauze in a Soxhlet extractor, showed that only negligible quantities of the carboxylic acids present in the secretion were left unextracted by this cold extraction method, which was adopted for the extraction of the secretions of individual animals. Larger quantities were extracted in a Soxhlet extractor.

3.4 Reference compounds

All the chemicals used for synthetic work were obtained from Aldrich Chemical Co., Merck, Sigma Chemical Co. or Holpro Co.

3.4.1 Preparation of 2,5-dimethylhexane and 2-methylheptane

Sodium (10.00 g, 0.435 mole) was carefully cut into small pieces and placed in a 500 ml flask fitted with an efficient double surface reflux condenser[1]. A mixture of pure 1-bromo-2-methylpropane (14.90 g, 0.109 mole) and 1-bromobutane (14.90 g, 0.109 mole), both compounds previously dried over anhydrous sodium sulphate, was added to the sodium in 5 ml volumes through the condenser. After each addition of the bromide mixture, the flask was heated gently, if necessary, to start the reaction and then shaken until no visible signs of a reaction taking place could be observed. After all the bromide mixture had been added, the reaction mixture was allowed to stand for 1 hour, with occasional shaking, and then rectified spirits (21.7 ml, 95% ethanol) was added by means of a dropping funnel through the condenser over a period of 1.5 hours. This was followed by aqueous ethanol (21.7 ml, 50% ethanol, 30 minutes) and distilled water (21.7 ml, 15 minutes). The mixture was now refluxed for 3 hours, whereafter water (330 ml) was added and the upper layer of crude alkane was separated from the aqueous layer. The crude alkane was washed once with an equal volume of water and dried over magnesium sulphate. The dried product was distilled and a clear liquid (5.32 g, 42.8%) collected at 105-130°C containing 2,5-dimethylhexane (26.2%, lit[2] bp 108°C), 2-methylheptane (45.2%, lit[2] bp 116°C) and octane (28.6%, lit[2] bp 125-127°C) according to GC-MS

analysis. No starting materials were detected. Mass spectra Figs. 3.1(a), 3.1(b) and 3.1(c).

3.4.2 Preparation of 3-methyl-3-buten-2-ol

2-Methyl-2-propenal (methacrolein) (0.21 g, 3.060 mmole, 9.8% pure) was placed in a 2 ml Reacti-Vial and methylmagnesium bromide (1.04 g, 3.0 M solution in diethyl ether) was added dropwise while the reaction mixture was magnetically stirred[3]. After each drop, the reaction was allowed to run to completion before the next drop was added. After all the methylmagnesium bromide had been added, water (1.40 ml) and 15% sulphuric acid (0.75 ml) were added successively, the magnetic stirrer bar was removed and a cap was placed on the vial. The reaction mixture was centrifuged for 2 minutes at 2500 r.p.m. to separate the organic and water layers. The ether layer was GC-MS analyzed directly and found to contain the title compound, 3-methyl-3-buten-2-ol (41.8%). Mass spectrum Fig. 3.2.

3.4.3 Preparation of 3,3-dimethylcyclohexanol

Methylmagnesium bromide (41.32 g, 3.0 M solution in diethyl ether) was cooled to 0°C in a 250 ml flask and copper(I)iodide (2.00 g, 0.0105 mole) was added to the solution[4]. 3-Methylcyclohex-2-enone (10.00 g, 0.0908 mole) was added to the cooled slurry at such a rate that the temperature did not rise above 5°C. The mixture was magnetically stirred for 1 hour and then quenched with saturated aqueous ammonium chloride (25 ml). Extraction of the aqueous layer with ether, followed by removal of the solvent, gave a yellow oil which was fractionally distilled at 170-173°C (lit[5] bp 74-76°C / 8 mm Hg) to give a fraction (2.42 g) containing 21.1% 3,3-dimethylcyclohexanone. Mass spectrum Fig. 3.3(a).

The ketone (2.30 g, 0.0182 mole) was reduced with lithium aluminium hydride (0.35 g, 9.22 mmole) in dry ether, followed by hydrolysis of the reaction products and unchanged lithium aluminium hydride with water (10 ml). Extraction of the aqueous layer with ether and removal of the solvent gave 3,3-dimethylcyclohexanol (2.07 g, 17.8%, calculated from starting material 3-methylcyclohex-2-enone), a yellow oil. Mass spectrum Fig. 3.3(b).

3.4.4 Preparation of verbenol

Verbenone (0.50 g, 3.33 mmole) was added to a solution of lithium aluminium hydride (0.063 g, 1.664 mmole) in ether (1 ml) and the reaction mixture magnetically stirred at room temperature for 1 hour in a Reacti-Vial[4]. The reaction products and unchanged lithium aluminium hydride were hydrolized by carefully adding water (1 ml) to the reaction mixture which was stirred for a further 30 minutes. The magnetic stir bar was removed, a cap was placed on the vial and the reaction mixture centrifuged for 2 minutes at 2500 r.p.m. to separate the organic and water layers. The ether layer was GC-MS analyzed directly and found to contain two isomers of verbenol (72.0% and 5.6%, respectively). Mass spectra Figs. 3.4(a) and 3.4(b), respectively.

3.4.5 Preparation of 2-methyl-2-propenal (methacrolein)

A two-necked 100 ml flask was equipped with a dropping funnel, the leg of which extended to the bottom of the flask, and a distillation head fitted with a thermometer and a condenser set for downward distillation[6]. To the condenser outlet was attached a 25 ml flask immersed in ice water. Sodium dichromate dihydrate (8.00 g, 0.0268 mole) was dissolved in water (42.8 ml) in an Erlenmeyer flask and concentrated sulphuric acid (5.7 ml) added to the solution. 2-Methyl-2-propenol (5.66 g, 0.0785 mole) was placed in the 100 ml flask, heated to boiling (113-115°C), and the dichromate solution added *via* the dropping funnel during 20 minutes. The mixture was boiled vigorously to maintain steady distillation and heating was continued for a further 15 minutes after all the oxidizing agent had been added. The water layer was separated from the distillate and the organic material was dried over anhydrous sodium sulphate. The dried distillate was redistilled slowly and a light brown liquid (0.50 g) was collected at 67-71°C containing 9.8% 2-methyl-2-propenal. lit[2] bp 68-70°C. Mass spectrum Fig. 3.5.

3.4.6 Preparation of 6-methyl-2-heptanone

A three-necked flask was equipped with a thermometer, dropping funnel and condenser[7]. A chromic acid solution was prepared by dissolving sodium dichromate dihydrate (4.00 g, 0.0134 mole) in water (12.0 ml) and slowly adding concentrated sulphuric acid (2.9 ml). The solution was cooled and diluted to 20 ml with water. The chromic acid solution (19.2 ml) was added dropwise during 15 minutes to a vigorously magnetically stirred solution of 6-methyl-2-heptanol (5.00 g, 0.0384 mole) in ether (15.4 ml) at such a rate that the temperature could be kept between 25 and 30°C, and cooled with ice water if necessary. After stirring the reaction mixture for a further two hours at room temperature, the ether layer was removed and the dark green aqueous layer was extracted with four portions of ether. The combined ether extracts were washed with saturated sodium hydrogen carbonate and saturated sodium chloride solution, and dried over anhydrous sodium sulphate. The product was filtered, the ether removed and the residue fractionally distilled to give the title compound, 6-methyl-2-heptanone (2.85 g, 57.9%), bp 168-170°C, a colourless liquid. Mass spectrum Fig. 3.6.

3.4.7 Preparation of 2,3-dimethyl-2-butanol

A 500 ml two-necked flask was equipped with an efficient double surface condenser and a 100 ml dropping funnel, with calcium chloride guard-tubes on top of both the condenser and the funnel[8]. Care was taken that all parts of the apparatus were thoroughly dry. Magnesium turnings (5.00 g, 0.206 mole) and a magnetic stirrer were placed in the flask, together with pure and dry ether (15 ml) and dry 2-bromopropane (3.14 g, 0.0255 mole). The reaction was started by heating the reaction mixture and the addition of a crystal of iodine. A further quantity of dry 2-bromopropane (22.16 g, 0.180 mole) and ether (50 ml) were mixed in the dropping funnel and added dropwise to the magnetically stirred mixture at such a rate as to ensure gentle refluxing of the ether. After all the 2-bromopropane was added, the mixture was allowed to cool. A solution of dry acetone (11.95 g, 0.206 mole) in ether (16 ml) was added to the rapidly stirred Grignard compound *via* the dropping funnel. The mixture was allowed to stand overnight. The product was hydrolized by pouring the reaction mixture onto crushed ice (105 g) and the magnesium hydroxide

dissolved by the addition of 10% hydrochloric acid. The mixture was transferred to a separatory funnel, the ether layer removed and the aqueous layer extracted with three portions of ether. The combined ethereal solutions were dried over potassium carbonate, filtered, the ether distilled off and the residue fractionally distilled to give a colourless fraction (17.46 g), bp 118-122°C, containing 88.7% 2,3-dimethyl-2-butanol. lit[2] bp 120-121. Mass spectrum Fig. 3.7.

3.4.8 Preparation of 3-hexanol

A mixture of 3-hexanone (1.50 g, 0.0150 mole), rectified spirits (3.9 ml, 95% ethanol) and water (1.3 ml) were placed in a 50 ml two-necked flask fitted with an efficient double surface condenser and a thermometer dipping into the reaction mixture[9]. Small pieces of sodium (0.86 g, 0.0372 mole) were added through the condenser at such a rate that the temperature of the reaction mixture could be kept below 30°C. After the sodium had reacted completely, water (13.1 ml) was added and the mixture was cooled to about 15°C. The ether layer was removed and washed with diluted hydrochloric acid (1:1, 0.6 ml) and water (0.6 ml), and dried over potassium carbonate. The product was distilled through a fractionating column to give a colourless fraction (0.99 g) at 133-136°C, containing 52.7% of 3-hexanol (lit[2] bp 134.5-135.5°C) and 47.3% of the starting ketone. Mass spectrum Fig. 3.8.

3.4.9 Preparation of methyl 2-pentyl ether (2-methoxypentane)

Dry 2-pentanol (10.00 g, 0.113 mole) was placed in a 50 ml flask fitted with an efficient double surface reflux condenser and sodium (0.33 g, 0.0142 mole) was added in small pieces[10]. The mixture was heated under reflux until all the sodium had reacted and methyl iodide (2.01 g, 0.142 mole) was then added to the alkoxide solution through the condenser and from a dropping funnel, and the resulting mixture was refluxed for two hours. The crude product was distilled and a fraction containing the ether collected at 78-84°C. The 2-pentanol still present in the crude ether was removed by heating the crude ether under reflux for two hours with a large excess of sodium. The volatile organic material was distilled from the alkoxide. The fraction (2.71 g) boiling at 80-83°C was collected and contained 87.6% of the title compound, methyl 2-pentyl ether. Mass spectrum Fig. 3.9.

3.4.10 Preparation of 3-hydroxy-3-methyl-2-butanone

3-Bromo-3-methyl-2-butanone[11]*:* In a 50 ml flask, fitted with an efficient double surface reflux condenser, was placed 3-methyl-2-butanone (5.00 g, 0.0581 mole), dry carbon tetrachloride (12 ml) and *N*-bromosuccinimide (NBS) (10.35 g, 0.0581 mole). The mixture was heated under reflux for three hours, cooled to 0°C and the succinimide, which is insoluble in cold carbon tetrachloride, filtered off. Water was added to the filtrate and the carbon tetrachloride layer was separated and dried over sodium sulphate. Using a fractionating column, the carbon tetrachloride was distilled off and a fraction (3.98 g) boiling at 160-175°C was collected and contained 98.8% of 3-bromo-3-methyl-2-butanone and 1.2% of 1-bromo-3-methyl-2-butanone, respectively. Mass spectra Figs. 3.10(a) and 3.10(b), respectively.

3-Hydroxy-3-methyl-2-butanone[12]: A solution of 3-bromo-3-methyl-2-butanone (2.00 g, 0.0121 mole) and sodium formate (5.18 g, 0.0762 mole) in ethanol (31.2 ml, 85%) was heated to reflux for twelve hours. The ethanol was distilled off and the product was extracted with ether. The water layer was extracted three times with small volumes of ether, the combined ether extracts dried over sodium sulphate and the ether removed, leaving a light brown liquid (0.98 g) containing 48.6% of 3-hydroxy-3-methyl-2-butanone (lit[2] bp 140-141°C) and 2.6% of 1-hydroxy-3-methyl-2-butanone, respectively. Mass spectra Figs. 3.10(c) and 3.10(d), respectively.

3.4.11 Preparation of 3-hydroxy-2-pentanone

The procedure described for the preparation of 3-hydroxy-3-methyl-2-butanone (§ 3.4.10) was used to synthesize 3-bromo-2-pentanone which was subsequently converted to 3-hydroxy-2-pentanone. The starting materials were 2-pentanone (20.00 g, 0.232 mole) and NBS (41.38 g, 0.233 mole). Distillation of the bromoketone gave a fraction (27.34 g) boiling at 165-180°C and containing 76.0% of 3-bromo-2-pentanone, 21.6% of 1-bromo-2-pentanone and 2.4% of 2-pentanone. Removal of the solvent from the product obtained in the second part of the synthesis gave a brown fraction (5.21 g) containing 89.5% of 3-hydroxy-2-pentanone (lit[2] bp 147-148°C) and 10.5% of 1-hydroxy-2-pentanone, respectively. Mass spectra Figs. 3.11(a), 3.11(b), 3.11(c) and 3.11(d), respectively.

3.4.12 Preparation of 2-hydroxy-3-pentanone

The procedure described for the preparation of 3-hydroxy-3-methyl-2-butanone (§ 3.4.10) was used to synthesize 2-bromo-3-pentanone which was subsequently converted to 2-hydroxy-3-pentanone. The starting materials were 3-pentanone (2.38 g, 0.0277 mole) and NBS (4.93 g, 0.0277 mole). Distillation of the bromoketone gave a fraction (1.98 g) boiling at 170-185°C and containing 18.4% of 2-bromo-3-pentanone and 76.0% of 3-pentanone. Removal of the solvent from the product obtained in the second part of the synthesis gave a yellow fraction (0.41 g) containing 22.1% of 2-bromo-3-pentanone and 73.3% of 2-hydroxy-3-pentanone (lit[13] bp 152.5°C), respectively. Mass spectra Figs. 3.12(a) and 3.12(b), respectively.

3.4.13 Preparation of 4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one

A solution of sodium borohydride in alkaline methanol (*vide infra*) (100 ml) was added over a period of 20 minutes to a mixture of 4-oxoisophorone (50.00 g, 0.329 mole) and cerium chloride heptahydrate ($CeCl_3 \cdot 7H_2O$) (45.00 g, 0.121 mole) in methanol (150 ml) at –5 to 0°C[14]. The reaction mixture was stirred at the same temperature for 30 minutes, poured into cold 20% aqueous ammonium chloride solution, and the organic substances were extracted three times with ether. The ether layer was washed with saturated sodium hydrogencarbonate and brine, and dried over anhydrous magnesium sulphate. After removal of the solvent, a yellow oil (3.20 g) was left containing 31.3% of 4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one (lit[14] bp 109-111°C / 4 mm Hg, lit[15] bp 91-94°C / 0.003 mm Hg), 13.7% of 4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one (lit[14] bp 123-127°C / 4 mm Hg, lit[16] bp 105-110°C / 0.1 mm Hg) and 50.8% of unreacted 4-oxoisophorone (lit[2] bp 92-94 / 11 mm Hg), respectively. Mass spectra Figs. 3.13(a) and 3.13(b), respectively.

Borohydride solution[17]: Sodium hydroxide (1.00 g, 0.0250 mole) was dissolved in water (12.5 ml), diluted to 250 ml with absolute ethanol, and sodium borohydride ($NaBH_4$) (9.64 g, 0.255 mole) added to the stirred solution. Any undissolved solids were filtered off.

3.4.14 Preparation of 2-methylpentanoic acid

Concentrated hydrochloric acid (12.0 ml) was added dropwise to calcium hypochlorite [$Ca(OCl)_2$] (15.00 g, 0.105 mole, 70%) and water (119.7 ml) until a bright yellow colour was obtained[18]. A solution of tetrachloromethane (59.8 ml) and *tert*-butanol (89.8 ml) was then added to the reaction mixture. A solution of 2-methyl-1-pentanol (7.50 g, 0.0734 mole) in *tert*-butanol (29.9 ml) was added to the reaction mixture over a period of four minutes, followed by stirring the reaction mixture at room temperature for five hours. The organic layer was separated from the water layer and the water layer was extracted six times with tetrachloromethane. The combined organic layers were washed with saturated sodium metabisulphide until the yellow colour disappeared from the solution. The organic solvent was removed, the residue dissolved in ether (300 ml), and the solution extracted five times with saturated sodium carbonate solution. The basic layer was extracted repeatedly with ether to remove all the unsaponifiable organic compounds. The water layer was then acidified with hydrochloric acid (3.7 M) and the organic acid extracted six times with ether. The combined ether extracts were washed with sodium chloride and water and dried over anhydrous magnesium sulphate. After filtration of the product, the ether was removed and the residue distilled to give a colourless fraction (4.30 g), bp 192-197°C, containing 95.5% of 2-methylpentanoic acid (lit[13] bp 195-196°C) and 4.5% of unreacted 2-methyl-1-pentanol. Mass spectrum Fig. 3.14

3.4.15 Preparation of 2,3-dimethylbutanoic acid

Diethyl methylmalonate[19]*:* A 500 ml three-necked flask was fitted with an efficient double surface condenser and a dropping funnel, with the central neck closed with a stopper. Sodium pieces (5.00 g, 0.217 mole) were placed in the flask and super-dry ethanol[20] (50.62 g, 1.099 mole) in the separatory funnel. Calcium chloride guard-tubes were used on the condenser and the separatory funnel. About half of the ethanol was let out of the dropping funnel and the vigorous reaction was controlled by cooling the flask in ice-water. The rest of the ethanol was added slowly and the mixture refluxed until all the sodium had reacted. The stopper in the central neck of the flask was removed and replaced by a sealed mechanical stirrer. The sodium ethoxide solution was cooled to about 50°C and redistilled diethyl malonate

(34.83 g, 0.217 mole) was added slowly through the dropping funnel. Redistilled methyl iodide (30.87 g, 0.217 mole) was then added dropwise and the resulting reaction mixture was refluxed until it was neutral to moist lithmus. Most of the remaining ethanol was removed by distillation, the mixture was cooled to about 20°C and water (86.9 ml) was added. The upper crude ester layer was separated and the aqueous water layer was extracted with a small volume of ether. The combined ester and ether layers were dried over anhydrous sodium sulphate and the solvent removed to give a fraction (31.07 g) containing 85.0% of diethyl methylmalonate (lit[2] bp 198-199°C).

Diethyl isopropylmethylmalonate: This malonate ester was synthesized in the same manner as described for diethyl methylmalonate, the only difference being that diethyl methylmalonate (26.40 g, 0.152 mole) and 2-bromopropane (21.94 g, 0.178 mole) were used as reagents to give a fraction (23.86 g), bp 138-142°C / 120 mm Hg, containing 57.7% of diethyl isopropylmethylmalonate.

2,3-Dimethylbutanoic acid: A 500 ml three-necked flask was equipped with a dropping funnel, a mechanical stirrer and a reflux condenser, and a hot solution of potassium hydroxide (23.85 g, 0.425 mole) in water (23.9 ml) was placed in the flask. Diethyl isopropylmethylmalonate (13.76 g, 0.0636 mole) was added slowly through the dropping funnel to the stirred hydroxide solution and the mixture was refluxed until a test sample dissolved completely in water. The mixture was diluted with water (23.9 ml) and the formed ethanol distilled off to prevent the formation of ethyl 2,3-dimethylbutanoate when the mixture is acidified. To the cold residue in the flask was carefully added a cold solution of concentrated sulphuric acid (20.7 ml) in water (53.7 ml), after which the solution was refluxed for four hours. The upper layer containing the organic acid was separated and the aqueous portion was extracted four times with ether. The combined ether and acid layers were washed with water and dried over anhydrous sodium sulphate. After the ether had been removed on a rotary evaporator, the organic material was distilled to give a fraction (3.76 g), bp 189-194°C, containing 36.7% of 2,3-dimethylbutanoic acid (5.5% overall yield, calculated from diethyl malonate) (lit[13] bp 191.7°C). Mass spectrum Fig. 3.15.

3.4.16 Preparation of 2-ethylbutanoic acid

The procedure described for the preparation of 2-methylpentanoic acid (§ 3.4.14) was used to synthesize 2-ethylbutanoic acid. The starting materials were 2-ethyl-1-butanol (5.00 g, 0.0489 mole) and calcium hypochlorite (10.00 g, 0.0696 mole). After removal of the solvent, distillation of the residue gave a colourless fraction (3.13 g), bp 191-197°C, containing 98.2% of 2-ethylbutanoic acid (54.1% yield calculated from reactant 2-ethyl-1-butanol) (lit[2] bp 99-101°C / 18 mm). Mass spectrum Fig. 3.16.

3.4.17 Preparation of 1-phenylethyl 2-methylpropanaote

This procedure was carried out in a fume cupboard. A reflux condenser was fitted to a 100 ml flask with a rubber stopper[21]. The stopper also carried an inlet tube by means of which a current of hydrogen chloride could be introduced to the bottom of the flask. The top of the condenser was fitted with a calcium chloride tube. The hydrogen chloride gas was prepared by adding concentrated sulphuric acid from a dropping funnel to solid ammonium chloride and conducting the gas to the reaction flask *via* the inlet tube. A mixture of 2-methylpropanoic acid (1.96 g, 0.0223 mole) and 1-phenylethanol (3.40 g, 0.0279 mole) was gently heated in an oil bath. Hydrogen chloride was introduced into the mixture for two hours. The resulting reaction product was washed with water, extracted with ether and dried over anhydrous sodium carbonate. The ether was distilled off and the organic residue distilled to give a light yellow fraction (3.00 g), bp 241-245°C, containing 24.7% of the target compound, 1-phenylethyl 2-methylpropanoate. Mass spectrum Fig. 3.17.

3.4.18 Preparation of 2-phenylethyl esters

The procedure described for the preparation of 1-phenylethyl 2-methylpropanoate (§ 3.4.17) was used to synthesize the 2-phenylethyl esters of butanoic acid, 2-methylpropanoic acid, pentanoic acid, nonanoic acid, pentadecanoic acid and hexadecanoic acid. Mass spectra Figs. 3.18(a), 3.18(b) and 3.18(c), respectively.

3.4.19 Preparation of 3-methyl-3-butenyl acetate

A mixture of glacial acetic acid (24.00 g, 0.400 mole), 3-methyl-3-buten-1-ol (17.21 g, 0.200 mole) and concentrated sulphuric acid (0.4 ml) in a 100 ml flask was stirred magnetically at room temperature for 16 hours[22]. The mixture was washed with water (100 ml) and the upper layer of crude ester was removed and washed successively with water, saturated sodium hydrogencarbonate and water. The ester was dried over anhydrous sodium sulphate, filtered and low-boiling volatile compounds was removed on a rotary evaporator at room temperature. The remaining material (14.50 g) was GC-MS analysed and found to contain 98.2% of 3-methyl-3-butenyl acetate and 1.8% of unreacted 3-methyl-3-buten-1-ol. Mass spectrum Fig. 3.19.

3.4.20 Preparation of dimethyl phthalate

Phthalic acid (10.00 g, 0.0602 mole), methanol (11.60 g, 0.361 mole) and concentrated sulphuric acid (1 ml) were magnetically stirred at room temperature for 12 hours[22]. The reaction mixture was washed with saturated sodium hydrogencarbonate and water, and the organic material extracted with ether. The product was dried over anhydrous magnesium sulphate, filtered and the solvent removed to give organic material (9.84 g) containing 21.4% of dimethyl phthalate (lit[2] bp 282°C), 39.2% of phthalic anhydride (lit[2] bp 284°C) and 39.4% of phthalic acid. Mass spectrum Fig. 3.20.

3.4.21 Preparation of 6-hexanelactam

Cyclohexanone oxime[23]*:* Cyclohexanone (20.00 g, 0.204 mole) was added to a solution of hydroxylamine hydrochloride (17.00 g, 0.245 mole) in water (40.0 ml) and the mixture cooled in ice-water. A solution of anhydrous sodium carbonate (13.00 g, 0.123 mole) in water (40.0 ml) was slowly added to the stirred mixture while the mixture temperature was maintained at 20-25°C. The oxime precipitated rapidly. The mixture was stirred at intervals for 10 minutes and the oxime was filtered, sucked dry and dried in a vacuum desiccator over phosphorus pentoxide. The dried product was recrystallised from petroleum ether (bp 100-120°C) and dried over silica gel and

phosphorus pentoxide to give pure cyclohexanone oxime (13.12 g, 56.9%). mp 89-91°C. Mass spectrum Fig. 3.21(a).

6-Hexanelactam prepared by a Beckmann rearrangement[23]*:* Cyclohexanone oxime (6.00 g, 0.0530 mole) was added to cold 85% sulphuric acid (12 ml) in a 500 ml beaker. The mixture was heated until effervescence started and then the heating was stopped as soon as a vigorous reaction started. A second quantity (6.00 g, 0.0530 mole) of the oxime was subjected to the same procedure. The product of the two preparations were combined and cooled in an ice-salt mixture. Crushed ice (30 g) was added to the organic material. The reaction mixture was stirred and 25% aqueous potassium hydroxide added until the mixture was faintly alkaline to phenolphthalein, while constantly ensuring that the temperature of the reaction mixture stayed below 20°C. The precipitated potassium sulfate was filtered off under reduced pressure and washed with chloroform (22.5 ml). The filtrate and washings were combined, the chloroform layer removed and the aqueous layer extracted three times with chloroform. The combined chloroform extracts were dried over sodium sulphate, filtered and the chloroform distilled off. The organic residue was crystallized from petroleum ether (bp 60-80°C) and the crystalline material dried over silica gel and phosphorus pentoxide to give 6-hexanelactam (1.51 g, 12.6% calculated from the synthetic oxime). mp 70-72°C (lit[2] bp 136-138°C / 10 mm Hg). Mass spectrum Fig. 3.21(b).

3.4.22 Preparation of dipropenyl ether

A mixture of diallyl ether (5.00 g, 0.0509 mole), dimethyl sulfoxide (DMSO) (8.10 g, 0.104 mole) and potassium *tert*-butoxide (1.18 g, 0.0105 mole) was refluxed at 70°C for 48 hours[24]. The mixture was distilled at 90-106°C to give a fraction (4.87 g) containing 5.4% of (*Z*,*Z*)-, (*E*,*Z*)- and (*E*,*E*)-dipropenyl ether, in a ratio 1 : 0.14 : 0.002, and unreacted allyl ether (26.0%) (lit[22] bp 93.5, 98.5 and 101.9°C, for respective isomers). Mass spectra Figs. 3.22(a), 3.22(b) and 3.22(c), respectively.

3.4.23 Preparation of Pummerer's ketone

An aqueous solution (1 litre) of pure *p*-cresol (10.81 g, 0.100 mole), potassium persulphate (27.03 g, 0.100 mole) and silver nitrate (1.70 g, 0.0100 mole) was stirred for 19 hours at 40°C[25]. Treatment of the ether-soluble product gave a yellow, crystalline, neutral fraction and a brown, resinous, alkali-soluble polycresol fraction. After one crystallisation from methanol, the neutral fraction yielded Pummerer's ketone (1.61 g, 15.0%) as colourless leaflets. mp 122-125°C. Mass spectrum Fig. 3.23.

3.4.24 Preparation of acetylcyclohexane

1-Cyclohexylethanol[8]: The procedure described for the preparation of 2,3-dimethyl-2-butanol (§ 3.4.7) was used to synthesize 1-cyclohexylethanol. The starting materials were magnesium turnings (4.00 g, 0.165 mole), cyclohexylchloride (2.36 g, 0.0199 mole) and dry acetaldehyde (7.20 g, 0.163 mole). After removal of the solvent, distillation gave a colourless fraction (16.71 g), bp 188-192°C, containing 80.0% of 1-cyclohexylethanol. Mass spectrum Fig. 3.24(a).

Acetylcyclohexane[7]: The procedure described for the preparation of 6-methyl-2-heptanone (§ 3.4.6) was used to synthesize acetylcyclohexane. The starting materials were 1-cyclohexylethanol (5.00 g, 0.0390 mole) and chromic acid solution (19.5 ml). The acetylcyclohexane (2.83 g, 57.5%) was distilled at 178-182°C. Mass spectrum Fig. 3.24(b).

3.4.25 Preparation of 3-methyl-2-hexanol

2-Methylpentanal[26]: A suspension of pyridinium chlorochromate (PCC) (21.08 g, 0.0978 mole) in dichloromethane (130 ml) was prepared in a 500 ml flask fitted with a reflux condenser. 2-Methyl-1-pentanol (6.66 g, 0.652 mole) in dichloromethane (13 ml) was added and the mixture was magnetically stirred for 1.5 hours and diluted with ether (130 ml). The supernatant solution was decanted from a black gum and the insoluble residue washed three times with ether. The combined organic solutions were passed through a layer of silica gel on a sinter filter

and the solvent removed. Distillation of the residual oil gave 2-methylpentanal (2.01 g, 30.1%), bp 118-121°C. Mass spectrum Fig. 3.25(a).

3-Methyl-2-hexanol[2]: 2-Methylpentanal (1.00 g, 0.0100 mmole) was stirred and treated dropwise with a solution of methylmagnesium bromide (3.96 g, 3.0 M solution in diethyl ether)[3]. After the addition of a drop of the Grignard solution, the reaction was allowed to run to completion before the next drop was added. After all the methylmagnesium bromide had been added, crushed ice (10.45 g) and 15% sulphuric acid (5.5 ml) were added successively to the reaction mixture. The ethereal solution was removed and the aqueous layer was extracted four times with ether. The combined ethereal solutions were dried over anhydrous potassium carbonate. Removal of the solvent, followed by fractional distillation gave 3-methyl-2-hexanol (0.58 g, 50.0%), bp 145-148°C. Mass spectrum Fig. 3.25(b).

3.4.26 Preparation of 1- and 3-acetyl-1-cyclohexene

A cold solution of cyclohexene (10.00 g, 0.122 mole) and acetic anhydride (73 ml) containing a few crystals of anhydrous zinc iodide was stirred magnetically for 15 minutes[26]. Zinc chloride (16.59 g, 0.122 mole) was added in one portion and the reaction mixture stirred at 0°C for a further two hours. The mixture was decomposed with ice, extracted with ether, the organic layers washed with 10% sodium hydroxide until the aqueous layer remained alkaline, and the ether removed *in vacuo*. The crude oil (11.32 g) was distilled and three fractions were collected at 180-190, 190-200 and 200-210°C, respectively. GC-MS analysis showed that the second fraction contained 1-acetyl-1-cyclohexene (lit[2] bp 201-202°C), 3-acetyl-1-cyclohexene and acetylcyclohexane (lit[2] bp 180-181°C) in a ratio of 1 : 0.17 : 0.25. Mass spectra Figs. 3.26(a) and 3.26(b), respectively.

3.4.27 Preparation of 4-hydroxy-3-hexanone

The procedure described for the preparation of 3-hydroxy-3-methyl-2-butanone (§ 3.4.10) was used to synthesize 4-bromo-3-hexanone which was subsequently converted to 4-hydroxy-3-hexanone. The starting materials were 3-hexanone (4.80 g, 0.0479 mole) and NBS (8.54 g, 0.0599 mole). Distillation of the bromoketone gave a fraction (4.14 g) boiling at 180-195°C and containing 71.2% of

4-bromo-3-hexanone, 11.2% of 2-bromo-3-hexanone and 0.9% of 3-hexanone. Removal of the solvent from the product obtained in the second part of the synthesis gave a brown material (1.82 g) containing 2-hydroxy-3-hexanone and 4-hydroxy-3-hexanone (lit[13] bp 132-135°C / 227 mm Hg). These two compounds elute within 3 seconds from each other, which makes it impossible to separate them by distillation. Mass spectra Figs. 3.27(a), 3.27(b), 3.27(c) and 3.27(d), respectively.

3.4.28 Preparation of methyl 2-hydroxy-2-methylpropanoate

2-Hydroxy-2-methylpropanoic acid (0.500 g, 4.803 mmole), methanol (0.310 g, 9.605 mmole) and one drop concentrated sulphuric acid were magnetically stirred at room temperature for 12 hours in a Reacti-Vial[22]. The reaction mixture was diluted with ether (1 ml), the magnetic stirrer bar removed, a cap placed on the vial and the contents of the vial centrifuged for 2 minutes at 2500 r.p.m. to separate the organic and water layers. The ether layer was removed with a syringe and analyzed by GC-MS which revealed that the reaction product contained 95.2% of methyl 2-hydroxy-2-methylpropanoate (lit[2] bp 137°C) and 4.8% of unreacted acid. Mass spectrum Fig. 3.28.

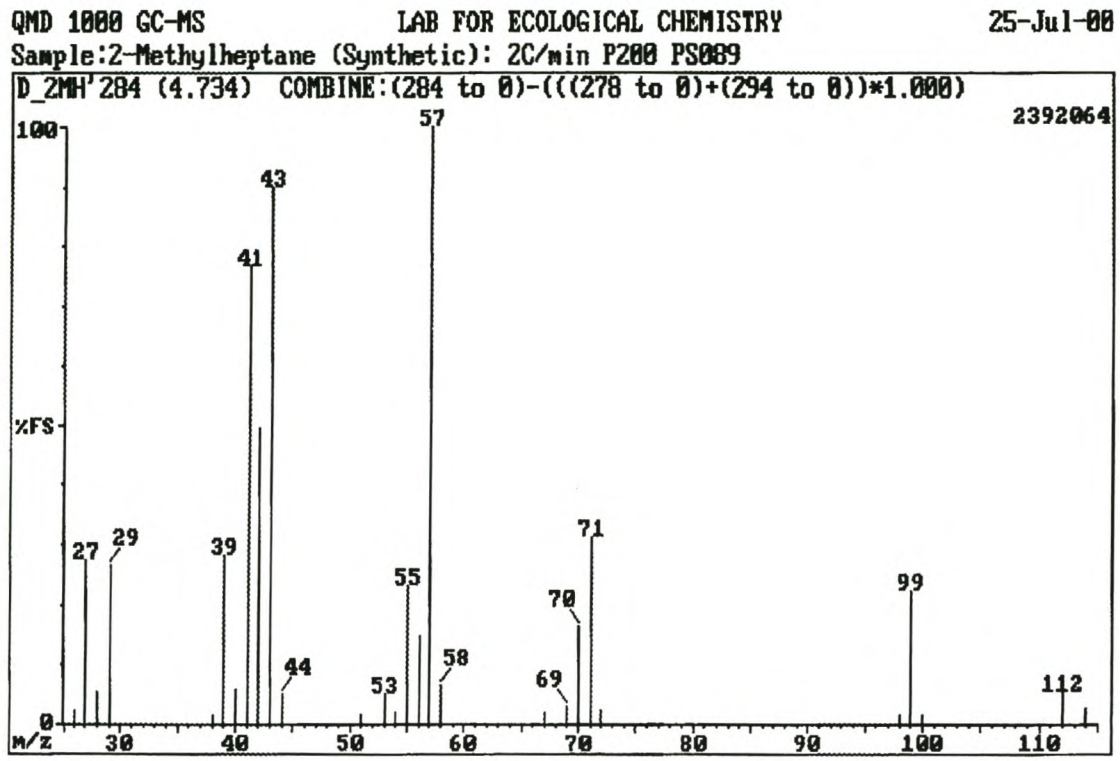

Fig. 3.1 (a): EI mass spectrum of 2,5-dimethylhexane

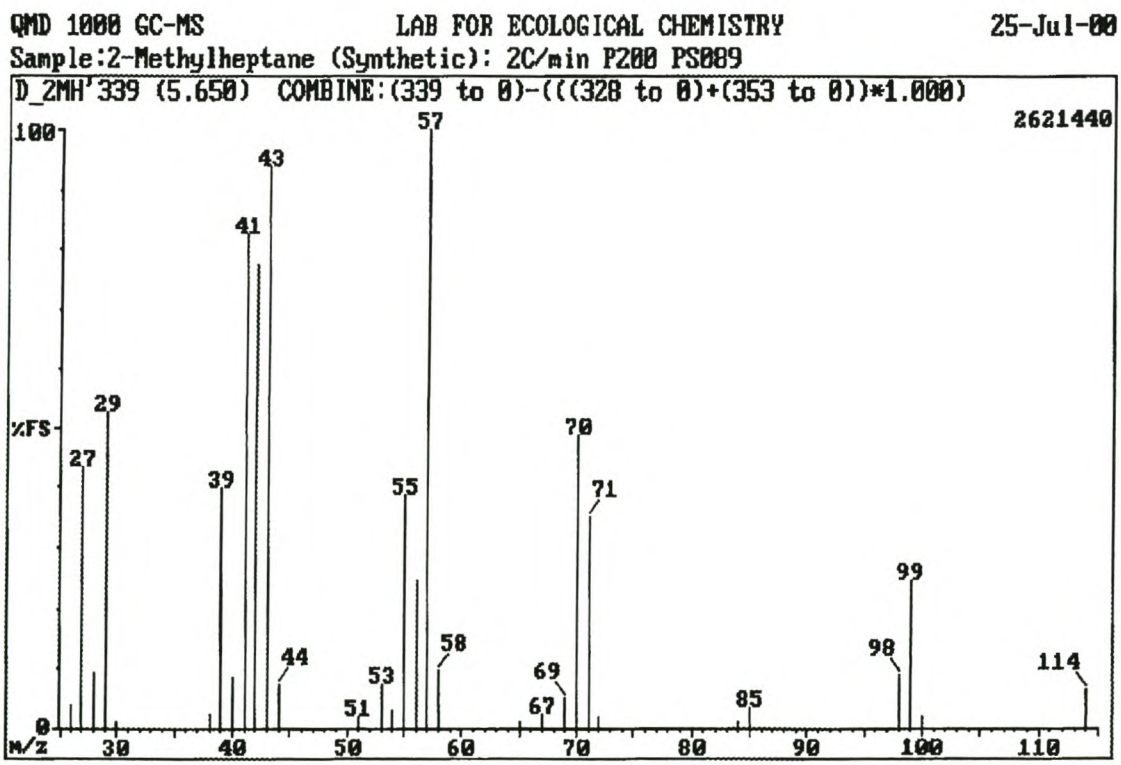

Fig. 3.1 (b): EI mass spectrum of 2-methylheptane

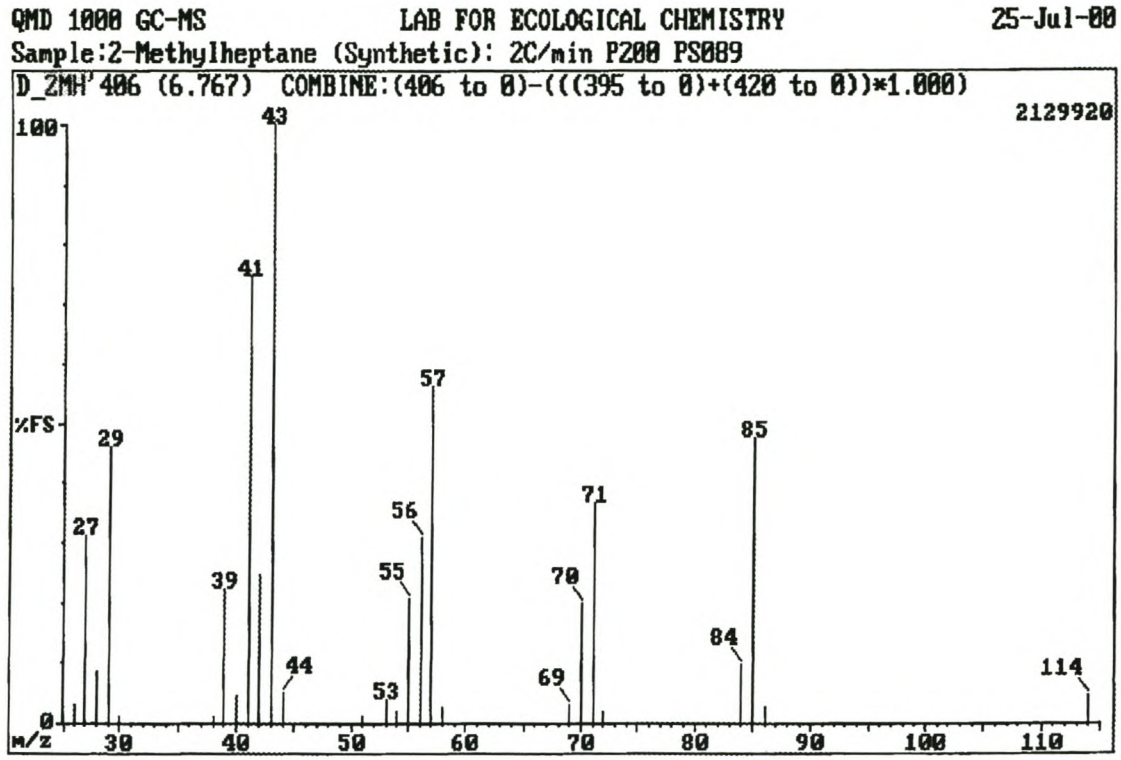

Fig. 3.1 (c): EI mass spectrum of octane

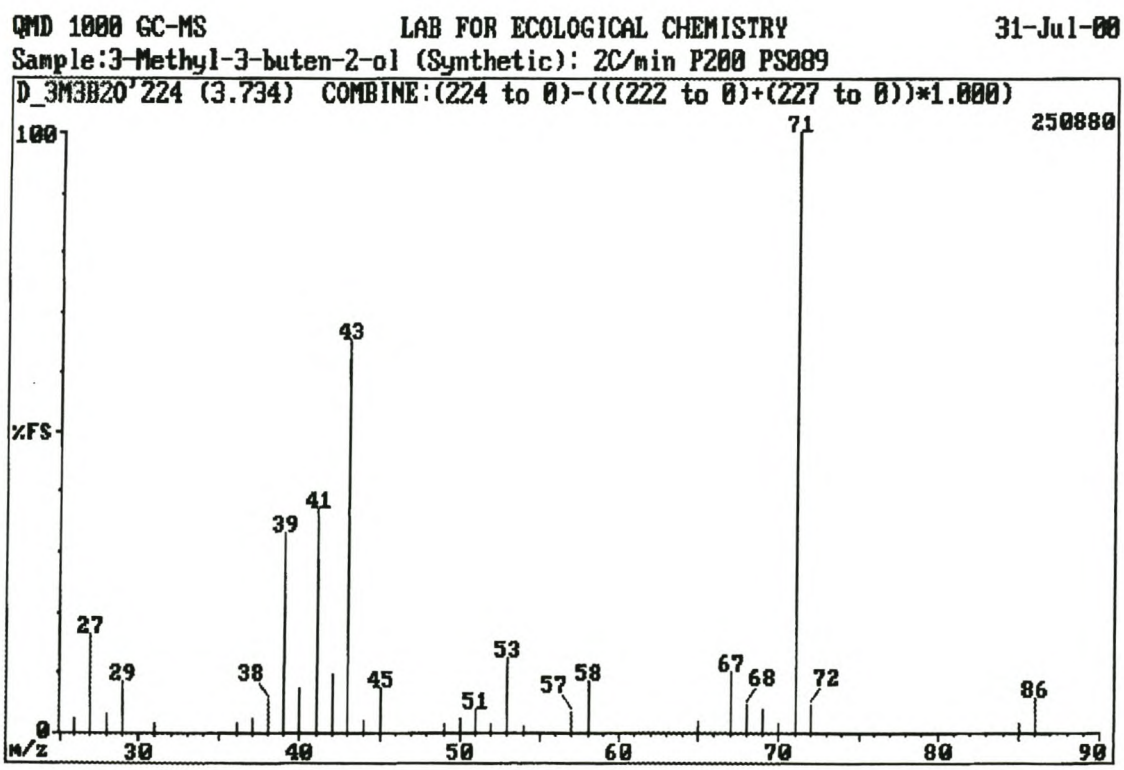

Fig. 3.2: EI mass spectrum of 3-methyl-3-buten-2-ol

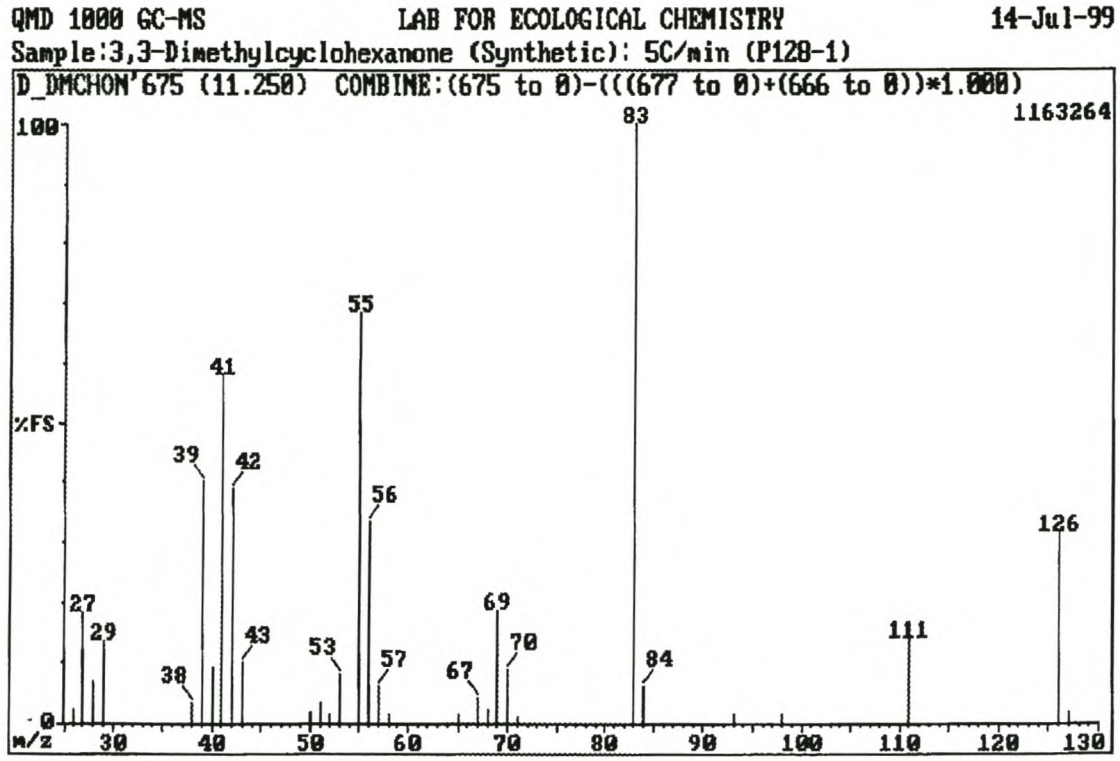

Fig. 3.3 (a): EI mass spectrum of 3,3-dimethylcyclohexanone

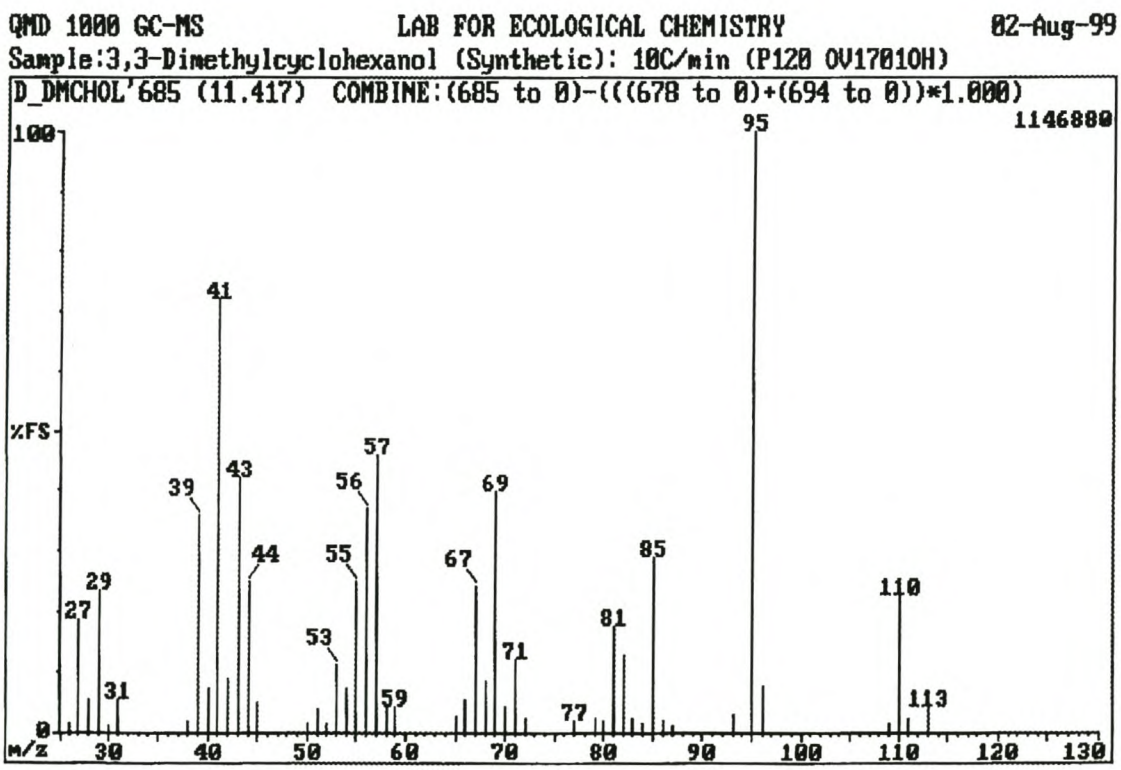

Fig. 3.3 (b): EI mass spectrum of 3,3-dimethylcyclohexanol

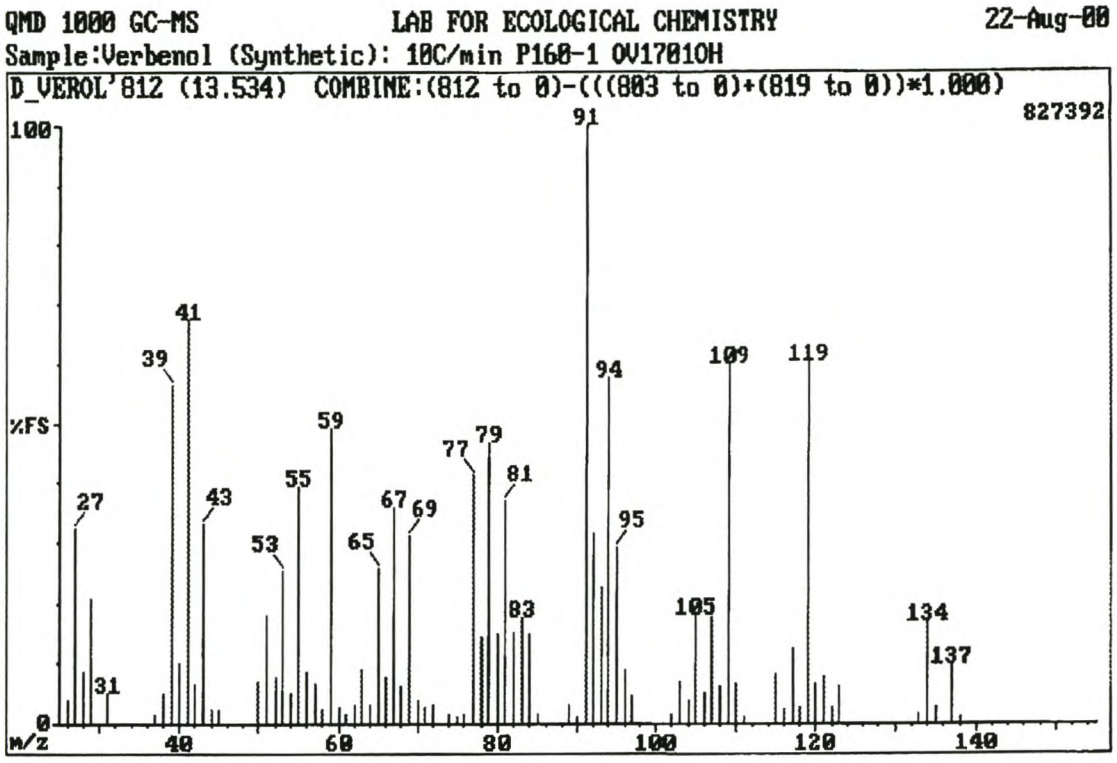

Fig. 3.4(a): EI mass spectrum of verbenol (first isomer)

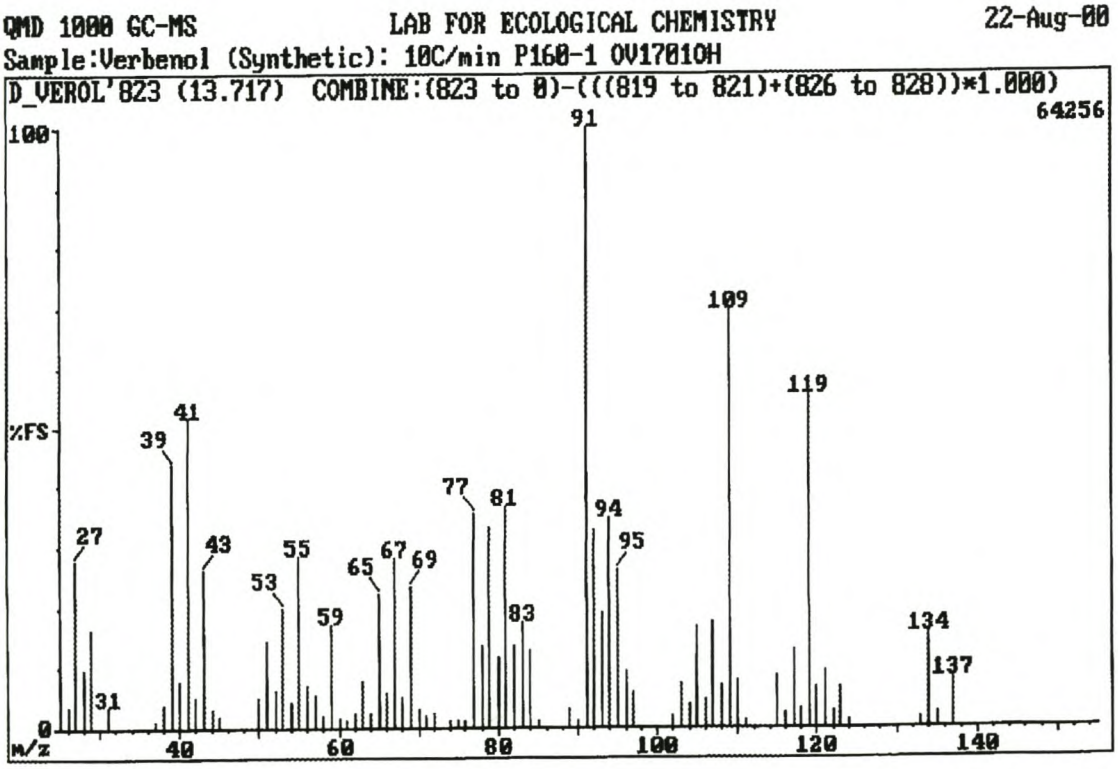

Fig. 3.4(b): EI mass spectrum of verbenol (second isomer)

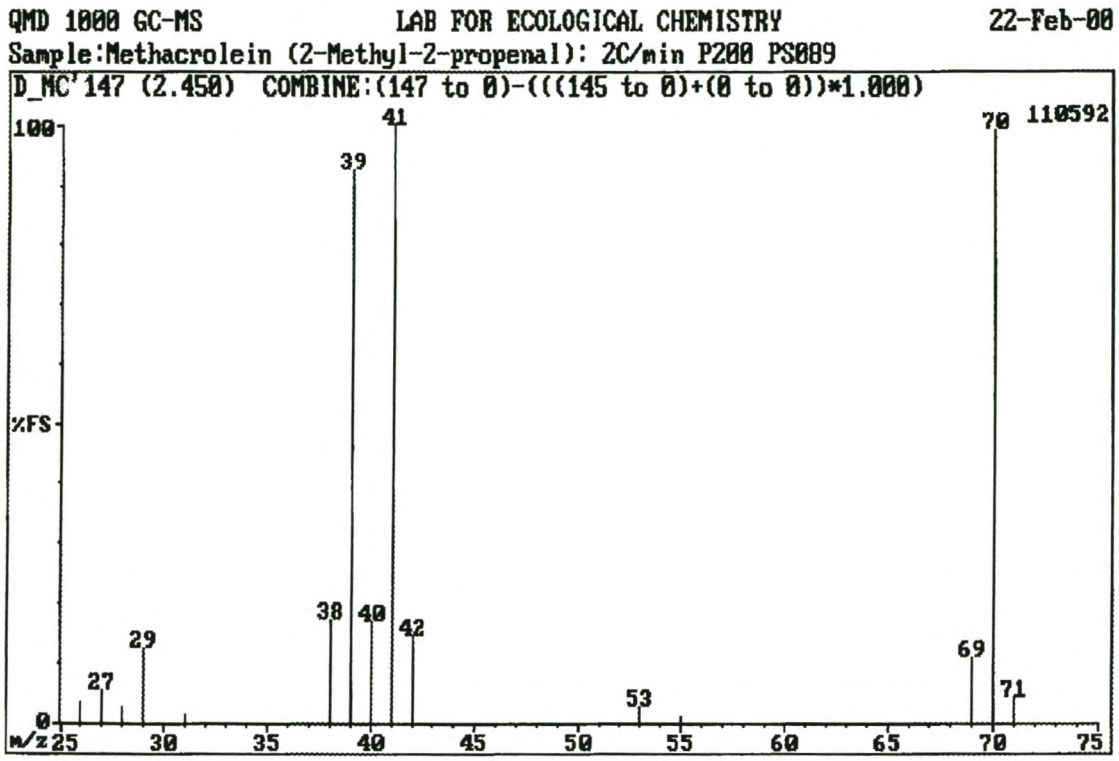

Fig. 3.5: EI mass spectrum of 2-methyl-2-propenal (methacrolein)

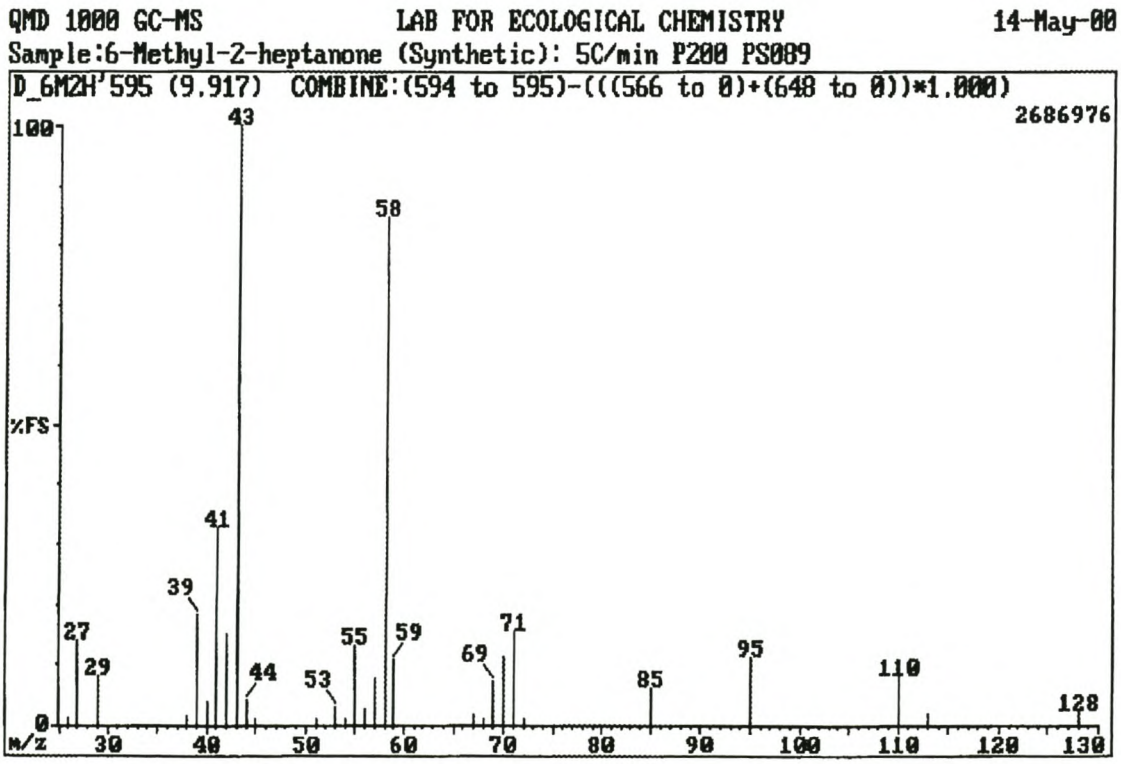

Fig. 3.6: EI mass spectrum of 6-methyl-2-heptanone

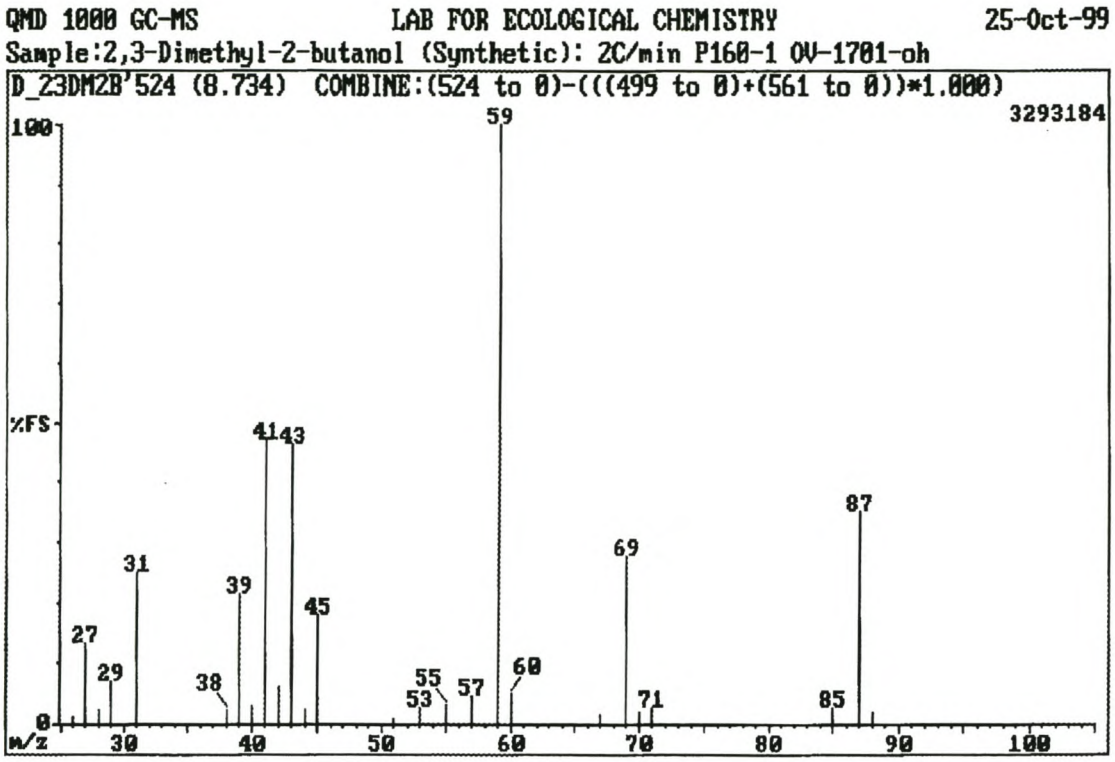

Fig. 3.7: EI mass spectrum of 2,3-dimethyl-2-butanol

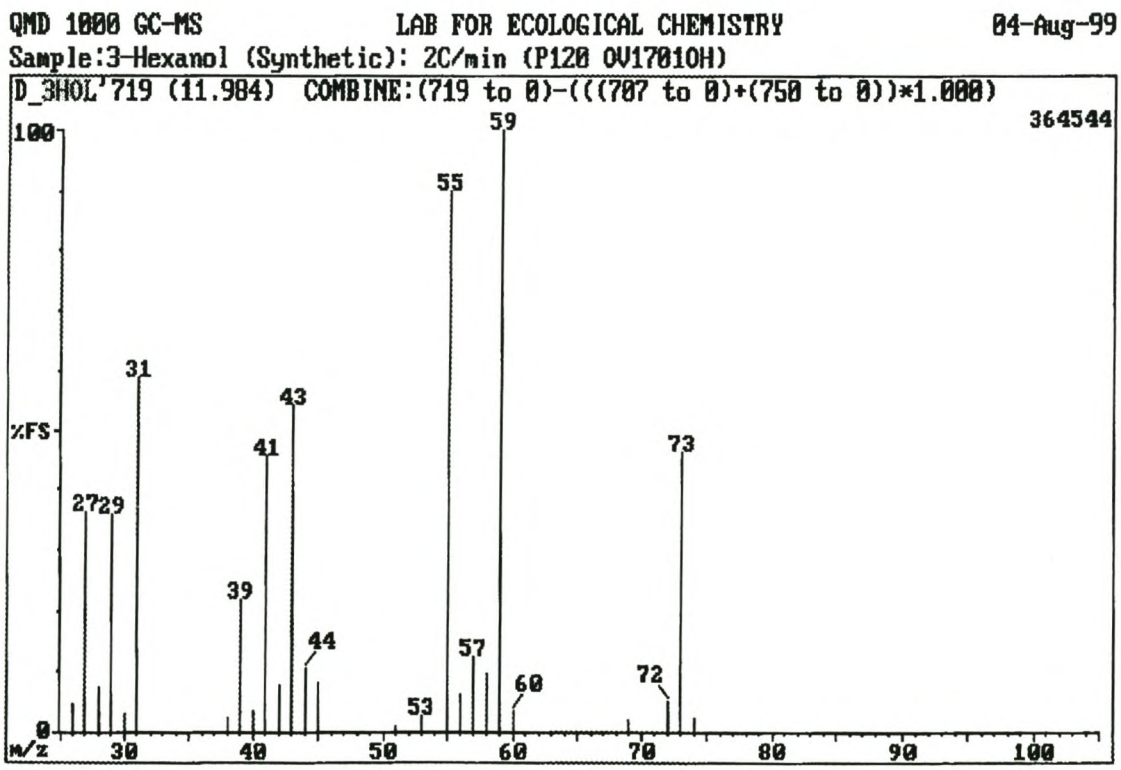

Fig. 3.8: EI mass spectrum of 3-hexanol

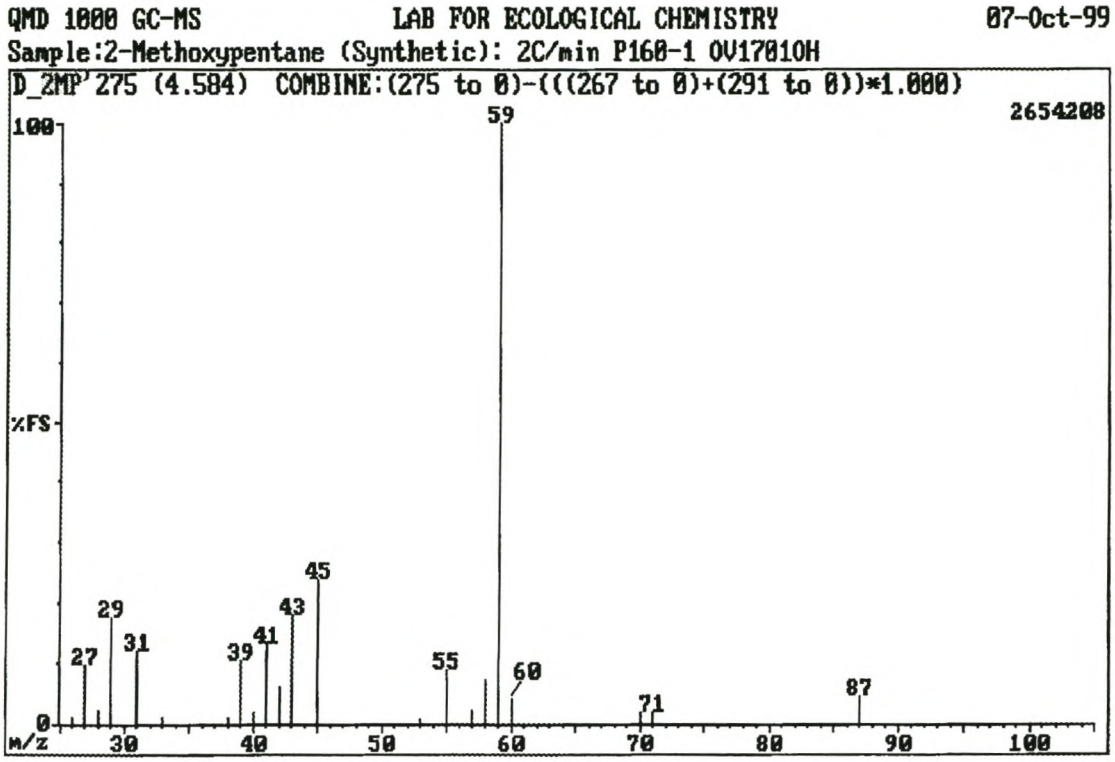

Fig. 3.9: EI mass spectrum of 2-pentyl ether (2-methoxypentane)

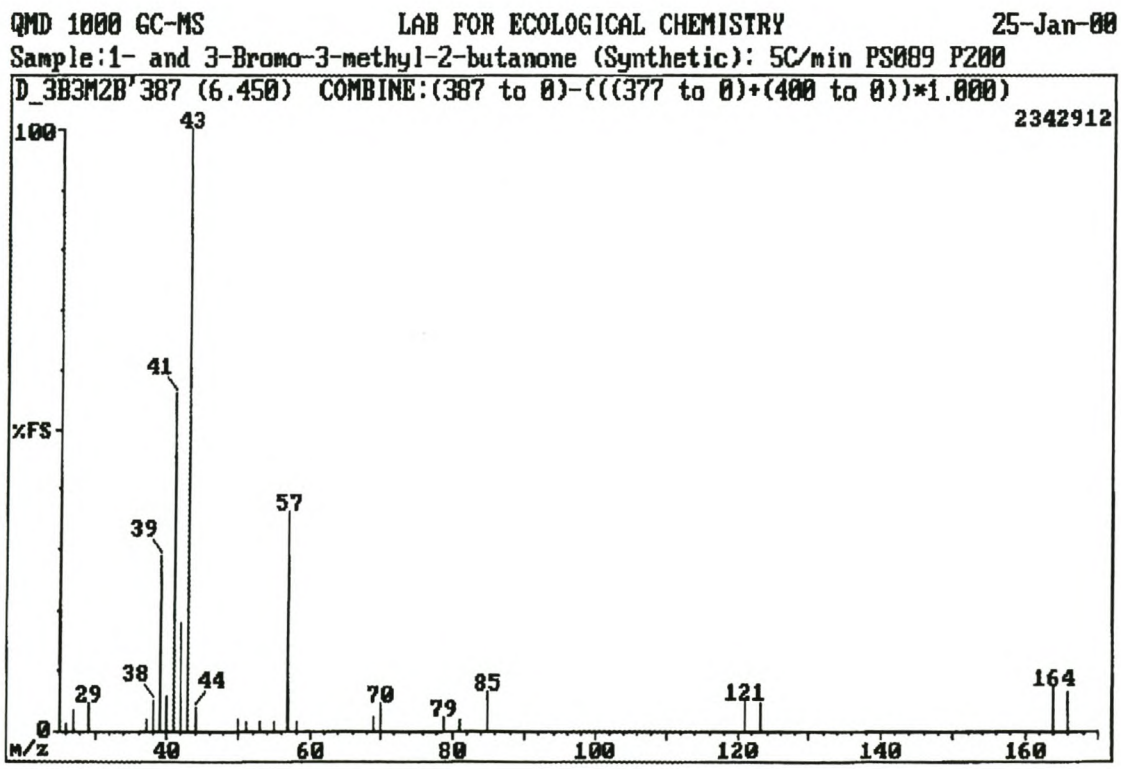

Fig. 3.10 (a): EI mass spectrum of 3-bromo-3-methyl-2-butanone

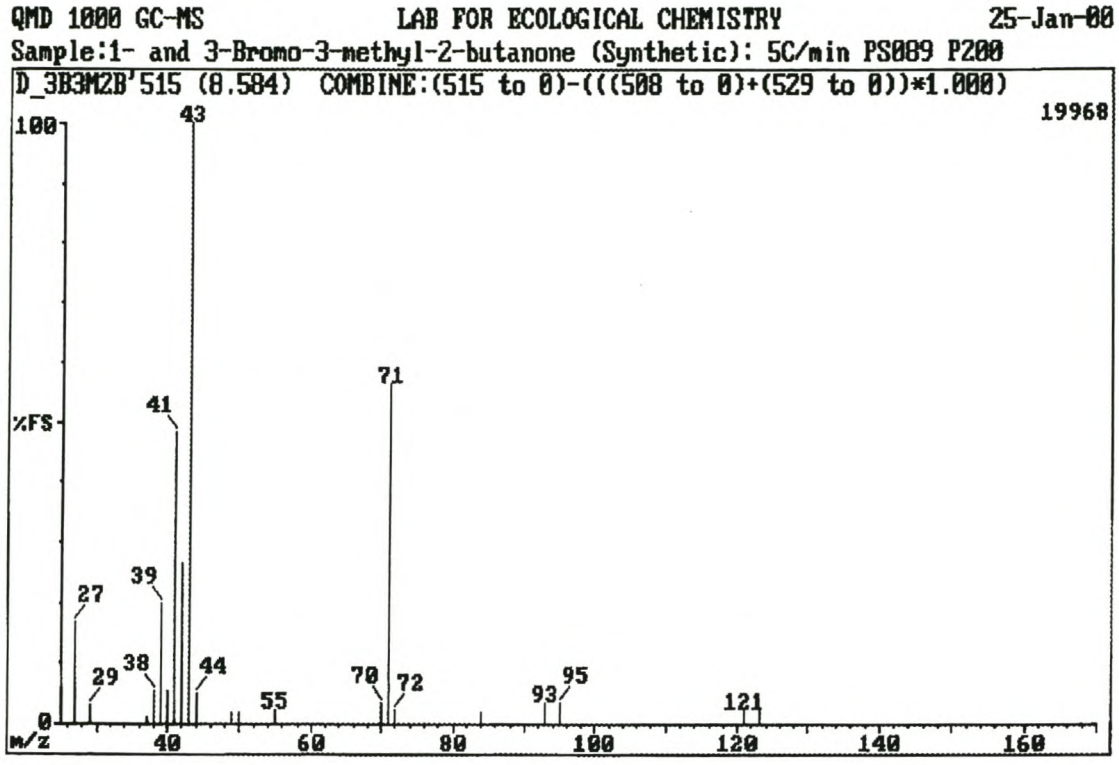

Fig. 3.10 (b): EI mass spectrum of 1-bromo-3-methyl-2-butanone

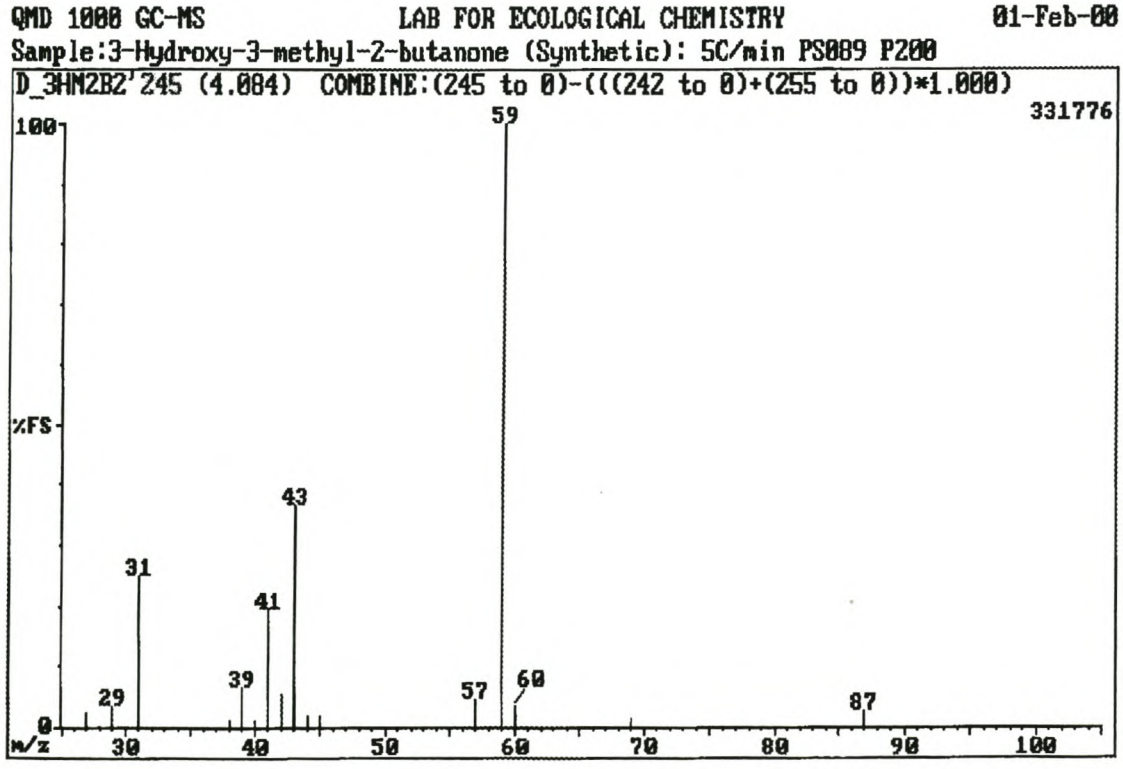

Fig. 3.10 (c): EI mass spectrum of 3-hydroxy-3-methyl-2-butanone

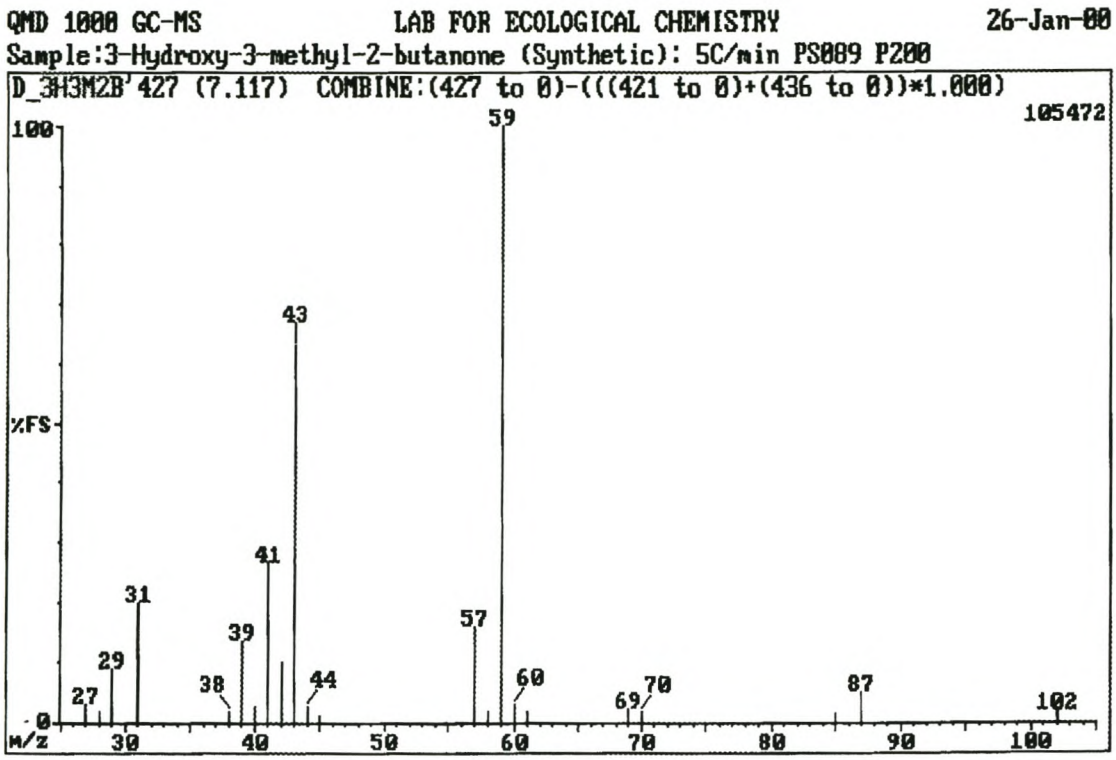

Fig. 3.10 (d): EI mass spectrum of 1-hydroxy-3-methyl-2-butanone

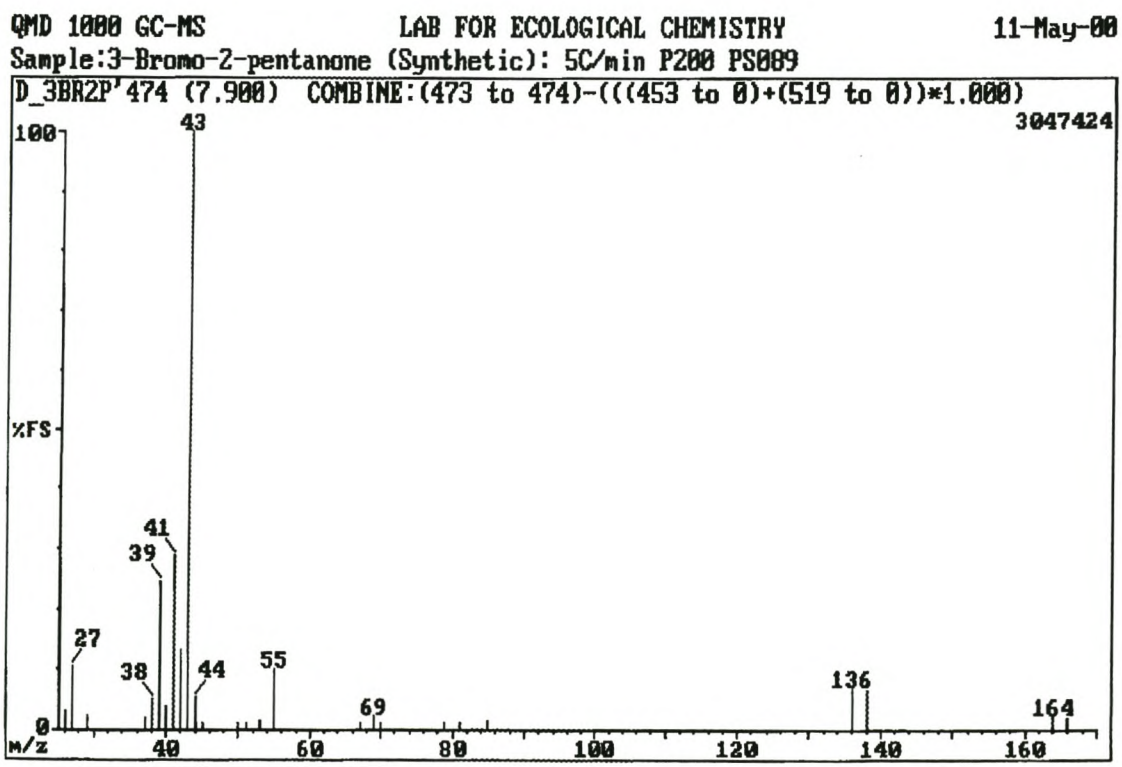

Fig. 3.11 (a): EI mass spectrum of 3-bromo-2-pentanone

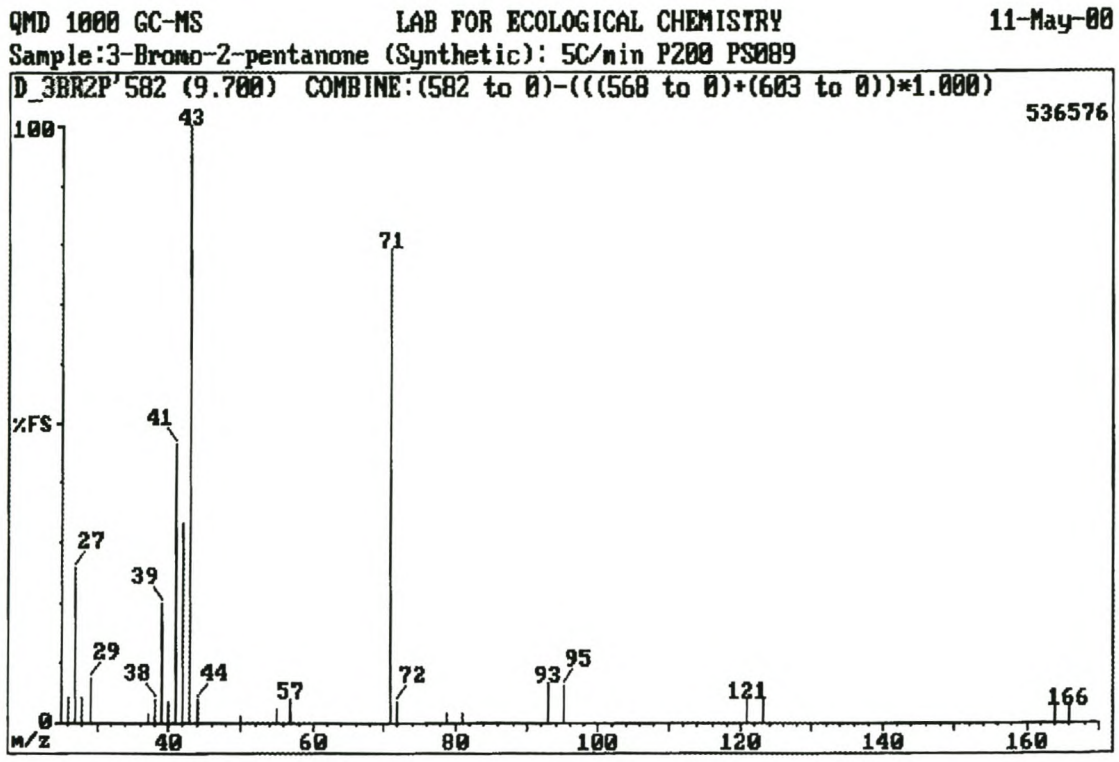

Fig. 3.11 (b): EI mass spectrum of 1-bromo-2-pentanone

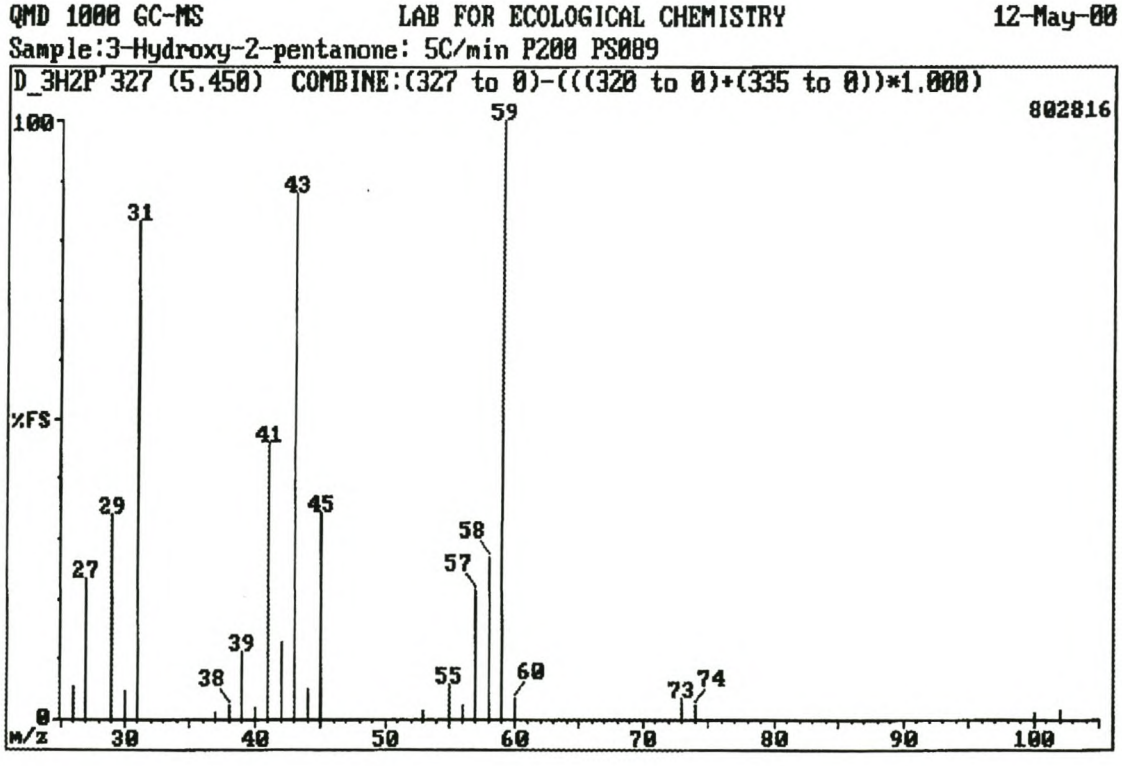

Fig. 3.11 (c): EI mass spectrum of 3-hydroxy-2-pentanone

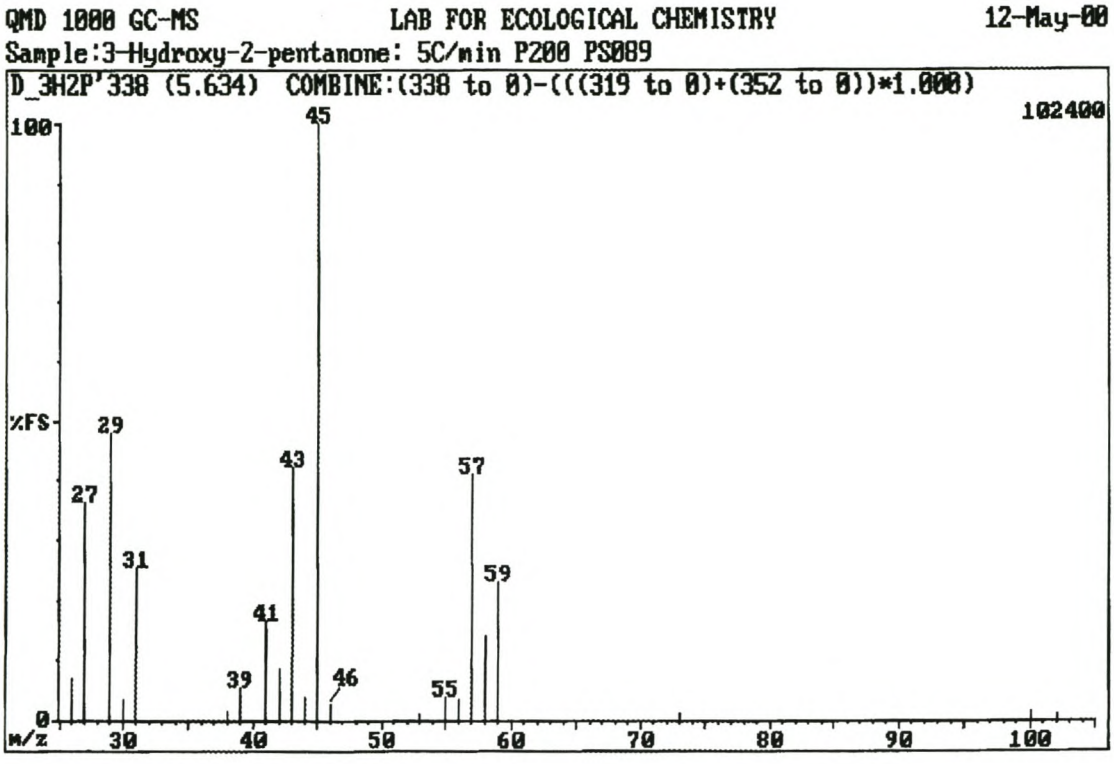

Fig. 3.11 (d): EI mass spectrum of 1-hydroxy-2-pentanone

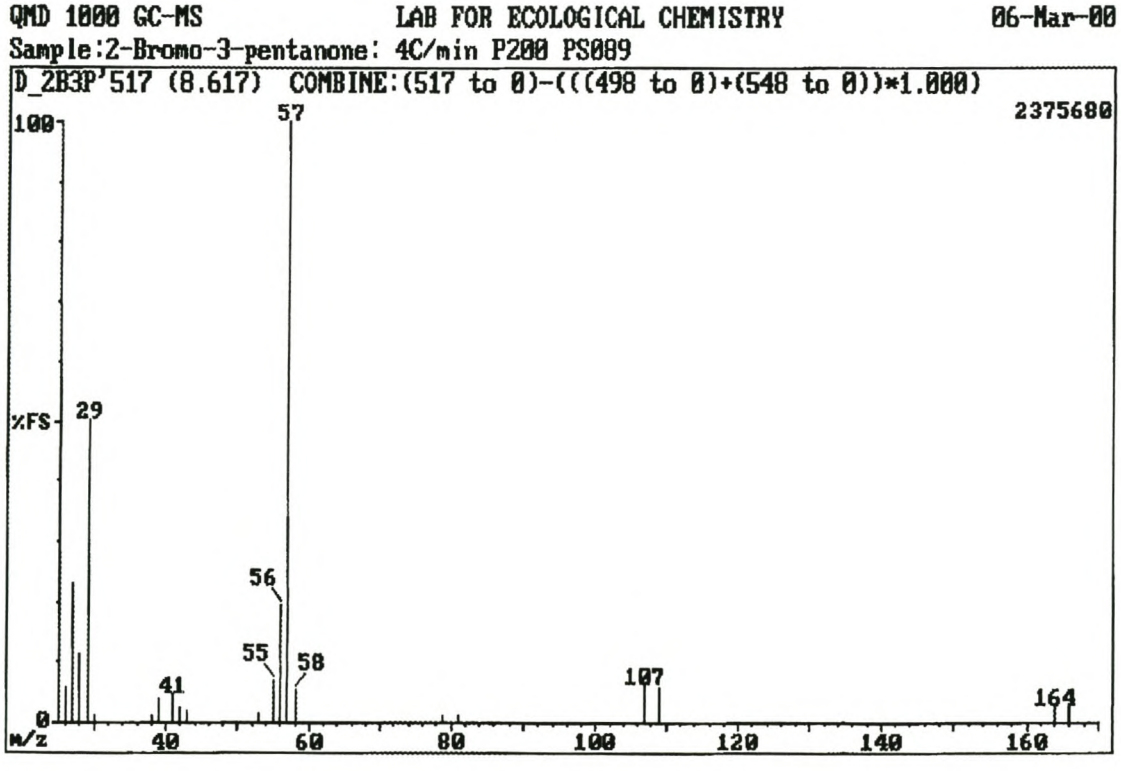

Fig. 3.12 (a): EI mass spectrum of 2-bromo-3-pentanone

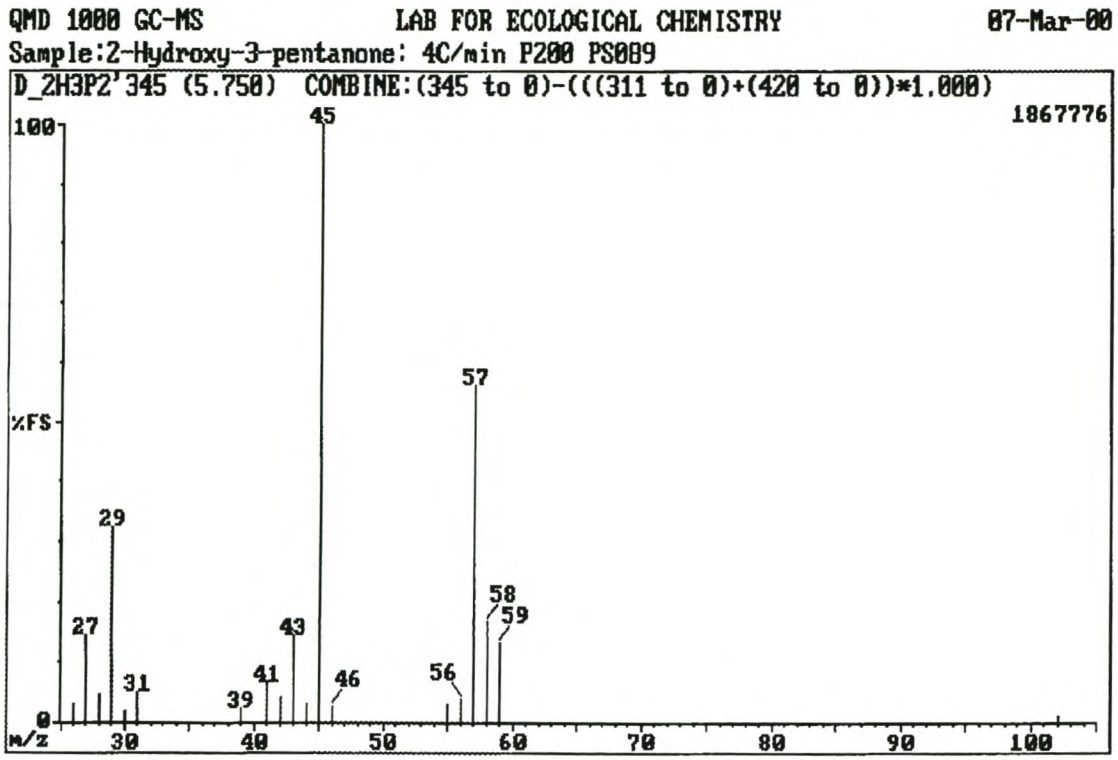

Fig. 3.12 (b): EI mass spectrum of 2-hydroxy-3-pentanone

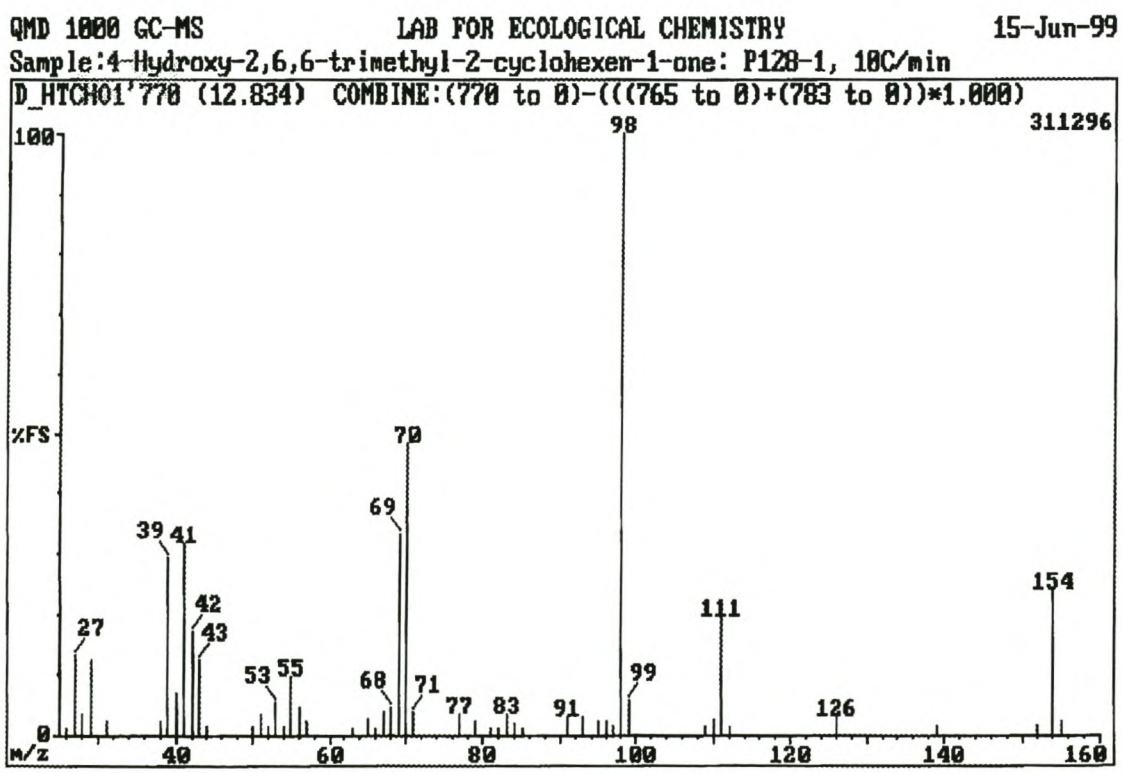

Fig. 3.13(a): EI mass spectrum of 4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one

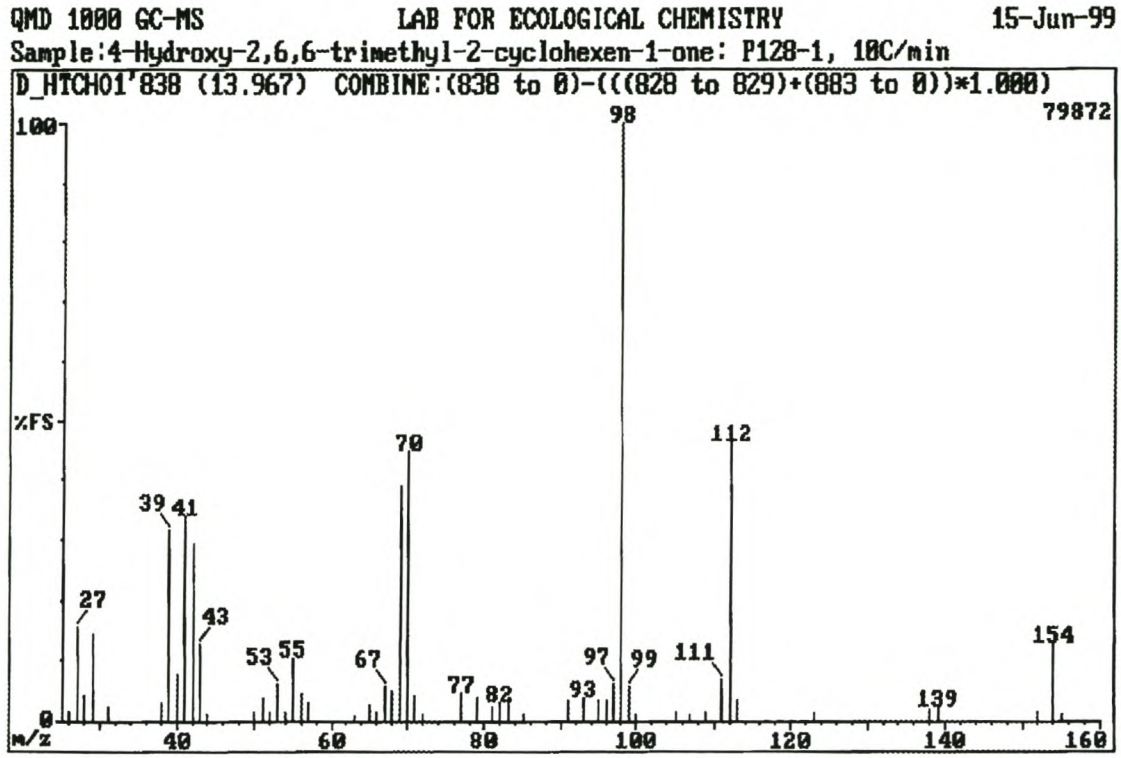

Fig. 3.13(b): EI mass spectrum of 4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one

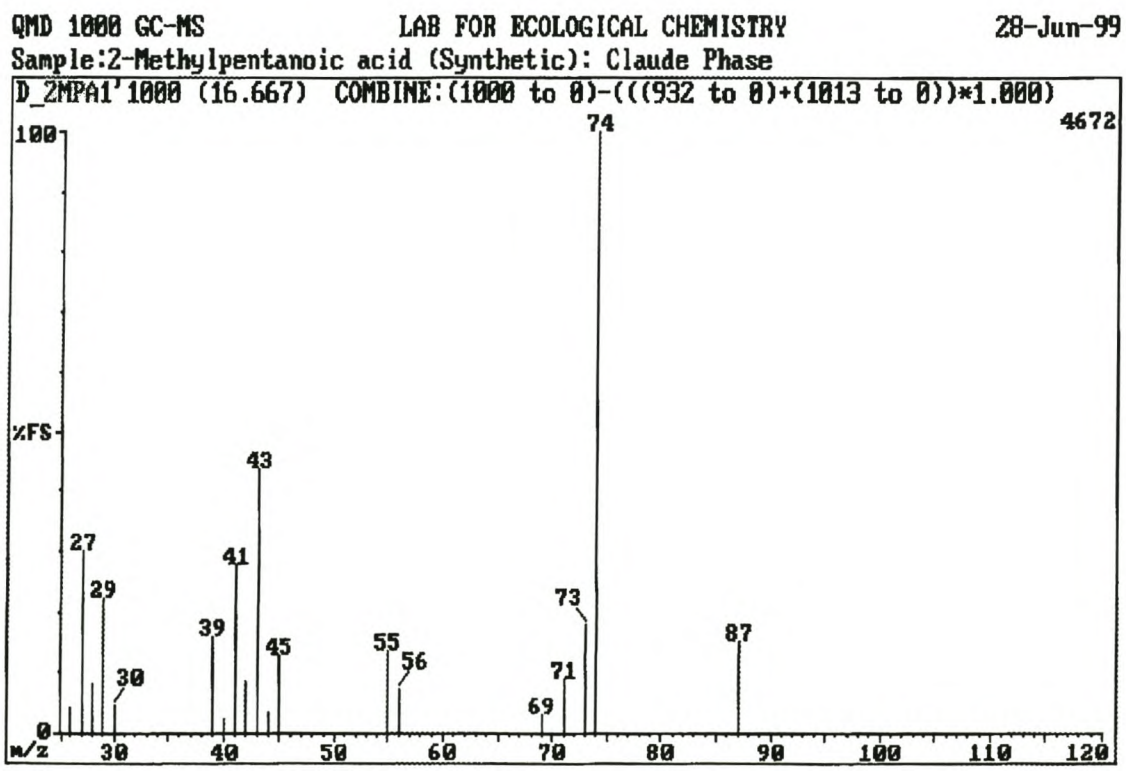

Fig. 3.14: EI mass spectrum of 2-methylpentanoic acid

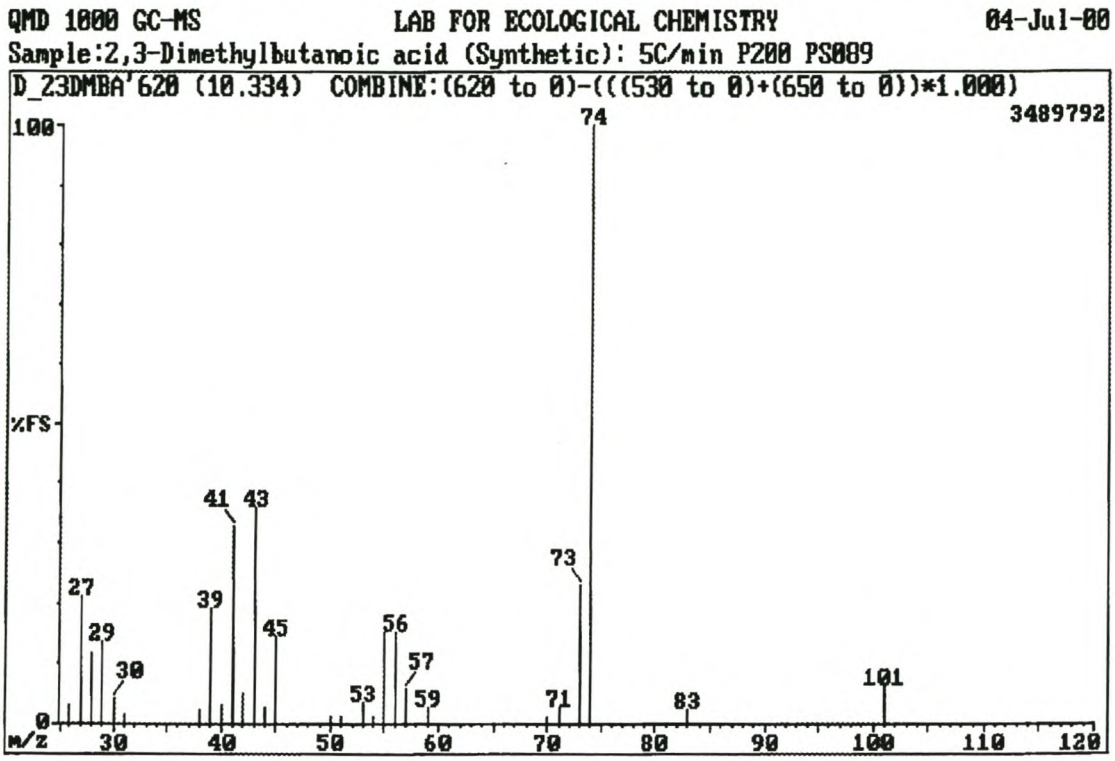

Fig. 3.15: EI mass spectrum of 2,3-dimethylbutanoic acid

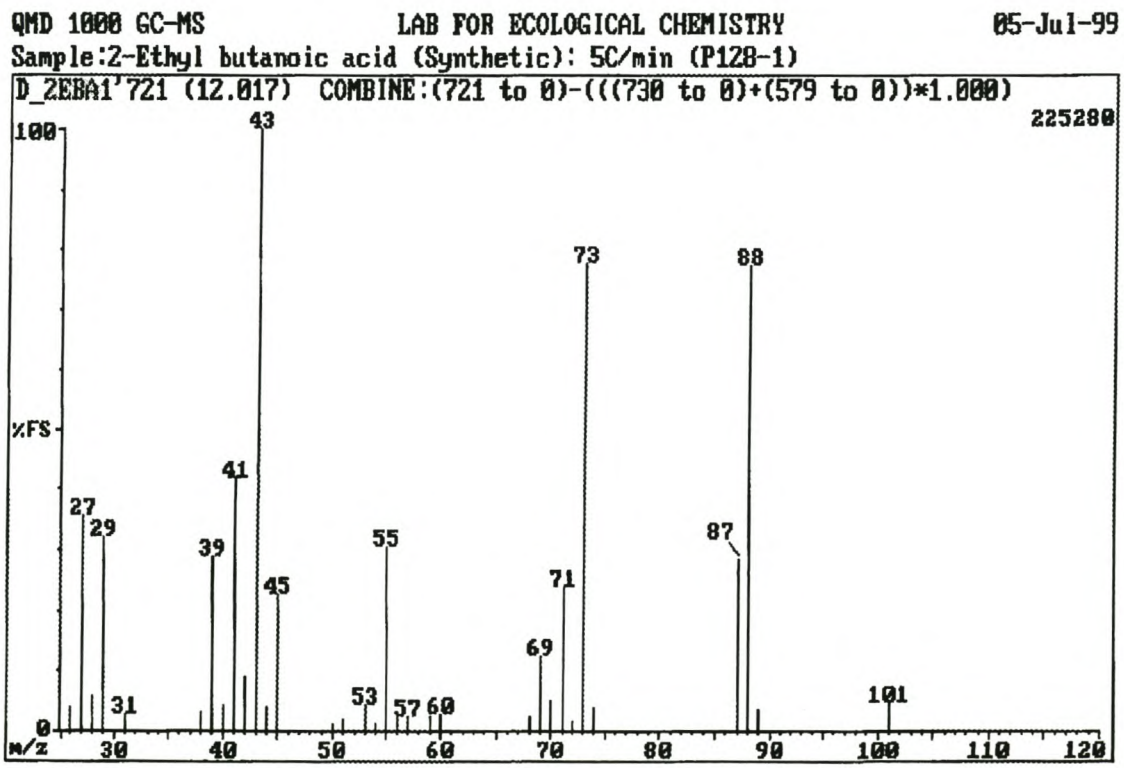

Fig. 3.16: EI mass spectrum of 2-ethylbutanoic acid

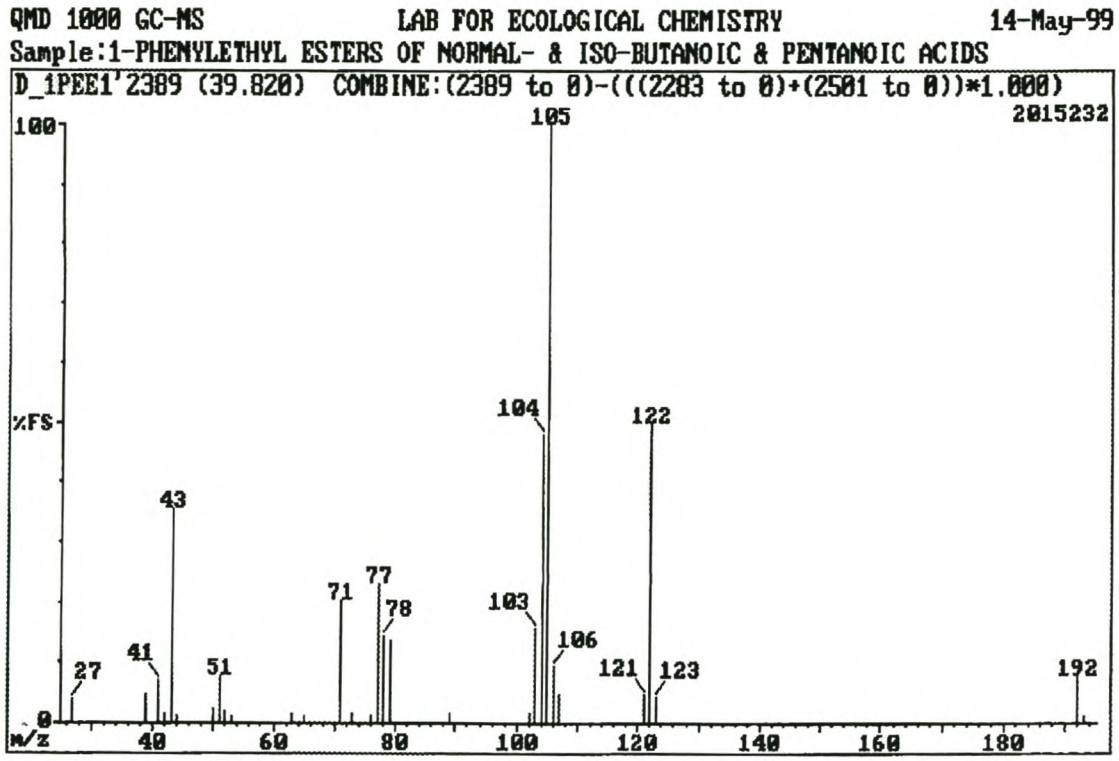

Fig. 3.17: EI mass spectrum of 1-phenylethyl 2-methylpropanoate

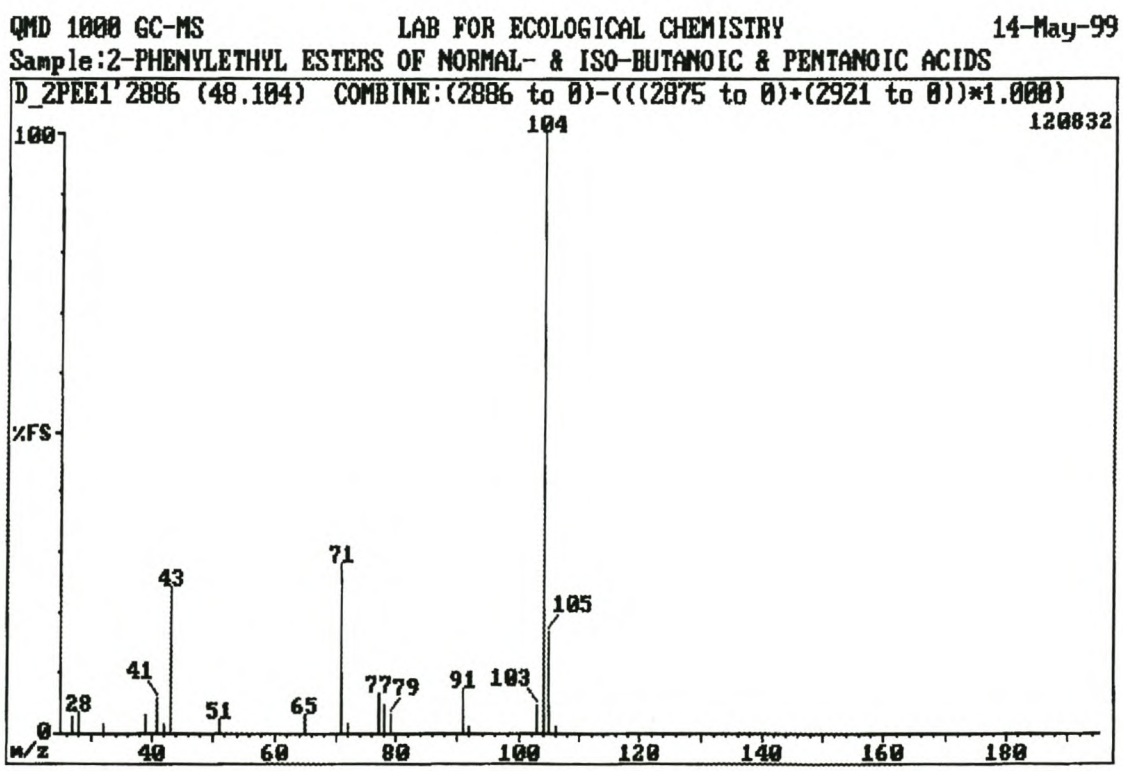

Fig. 3.18 (a): EI mass spectrum of 2-phenylethyl butanoate

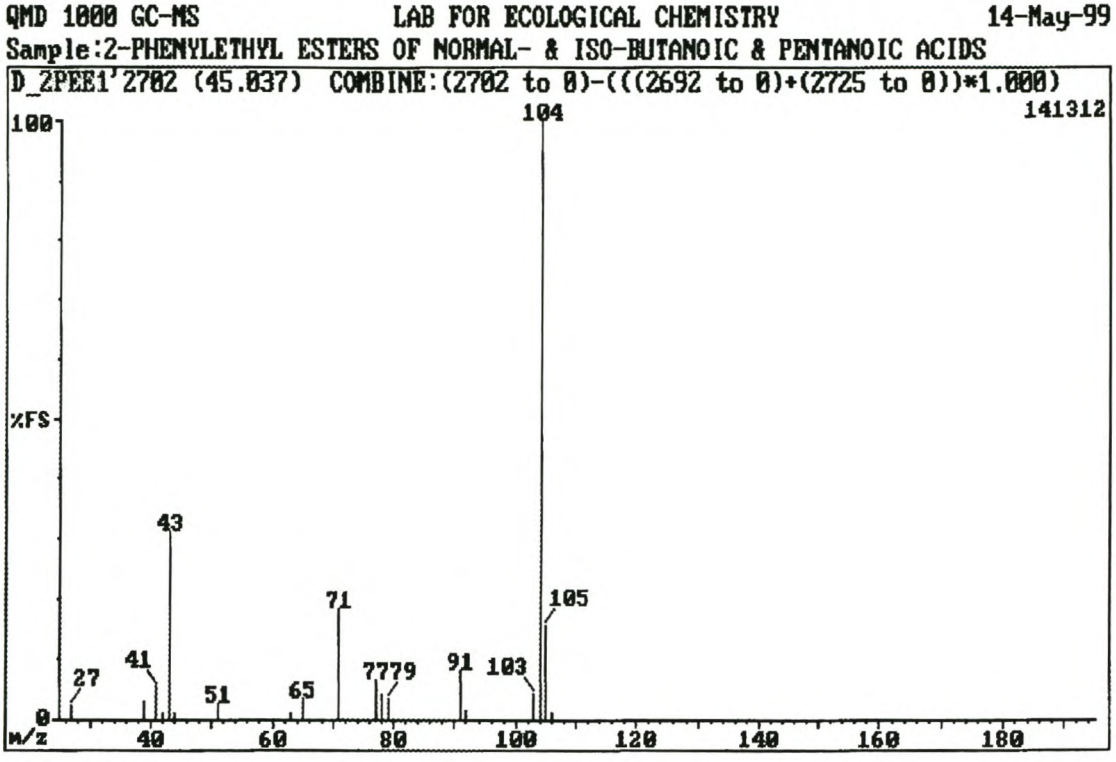

Fig. 3.18 (b): EI mass spectrum of 2-phenylethyl 2-methylpropanoate

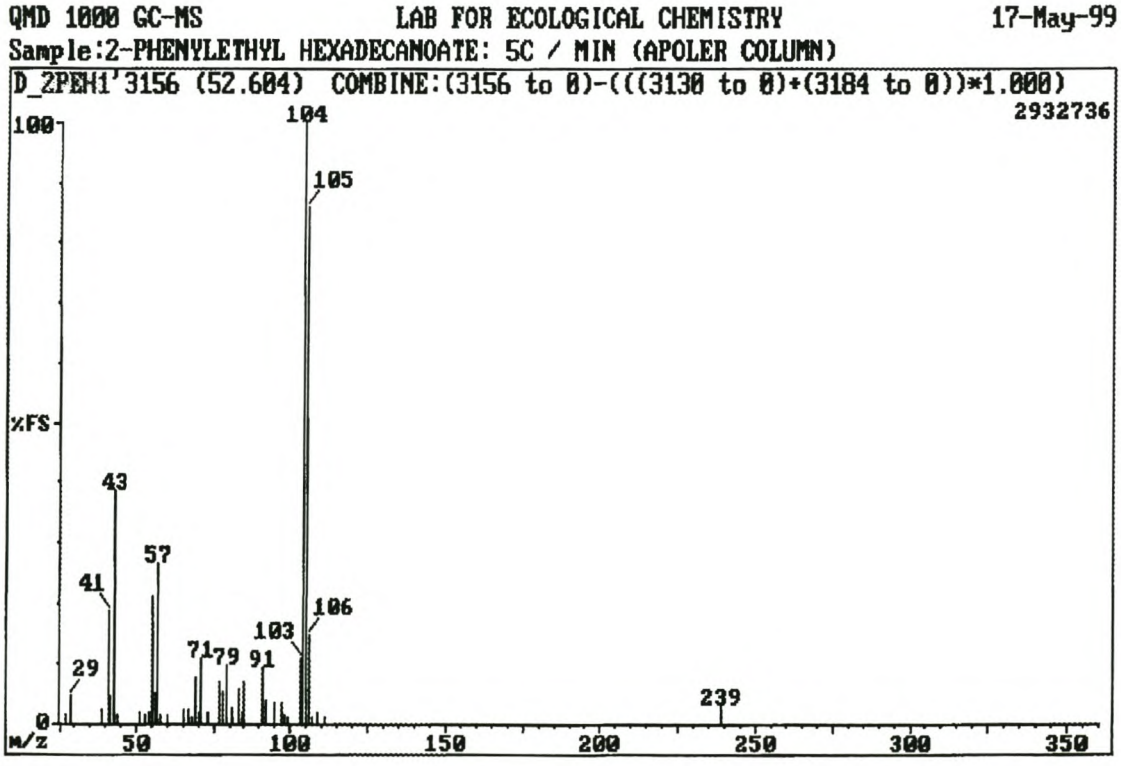

Fig. 3.18 (c): EI mass spectrum of 2-phenylethyl hexadecanoate

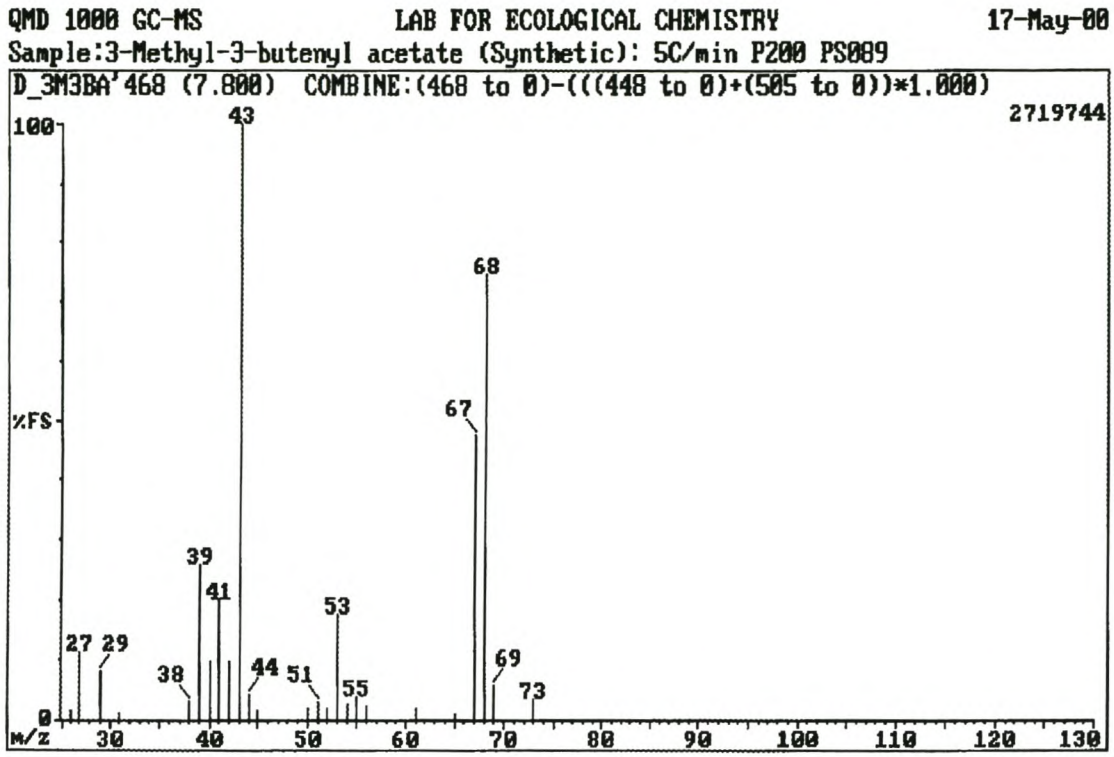

Fig. 3.19: EI mass spectrum of 3-methyl-3-butenyl acetate

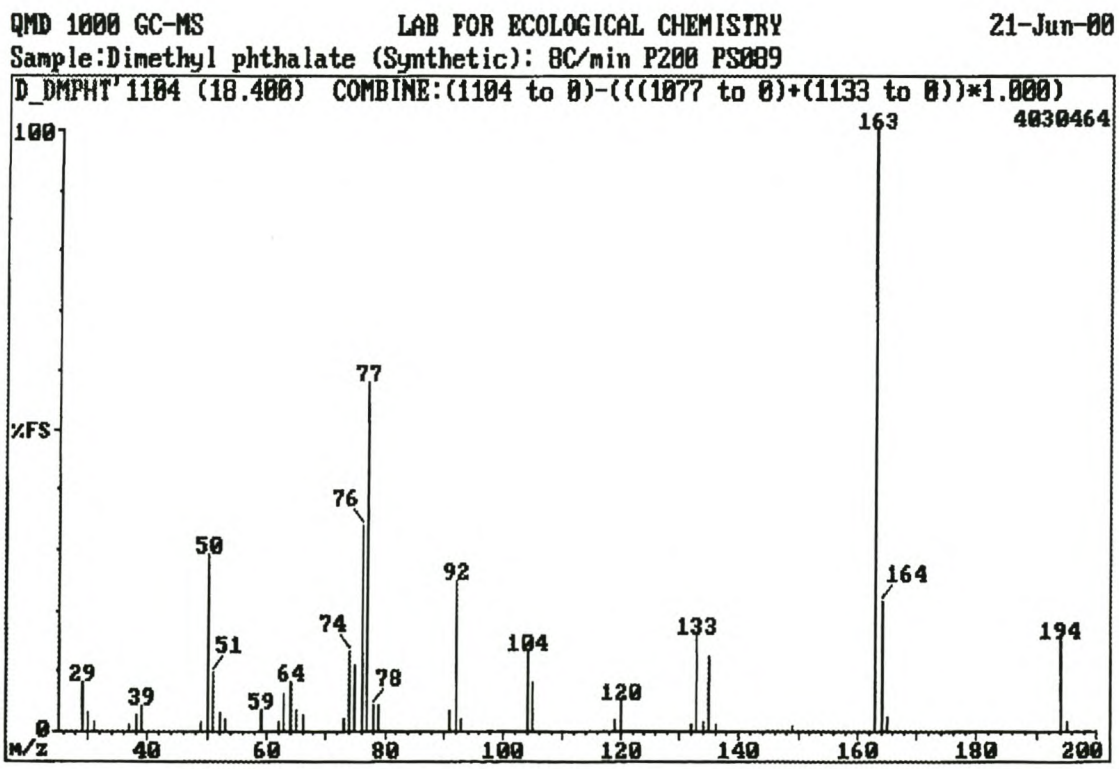

Fig. 3.20: EI mass spectrum of dimethyl phthalate

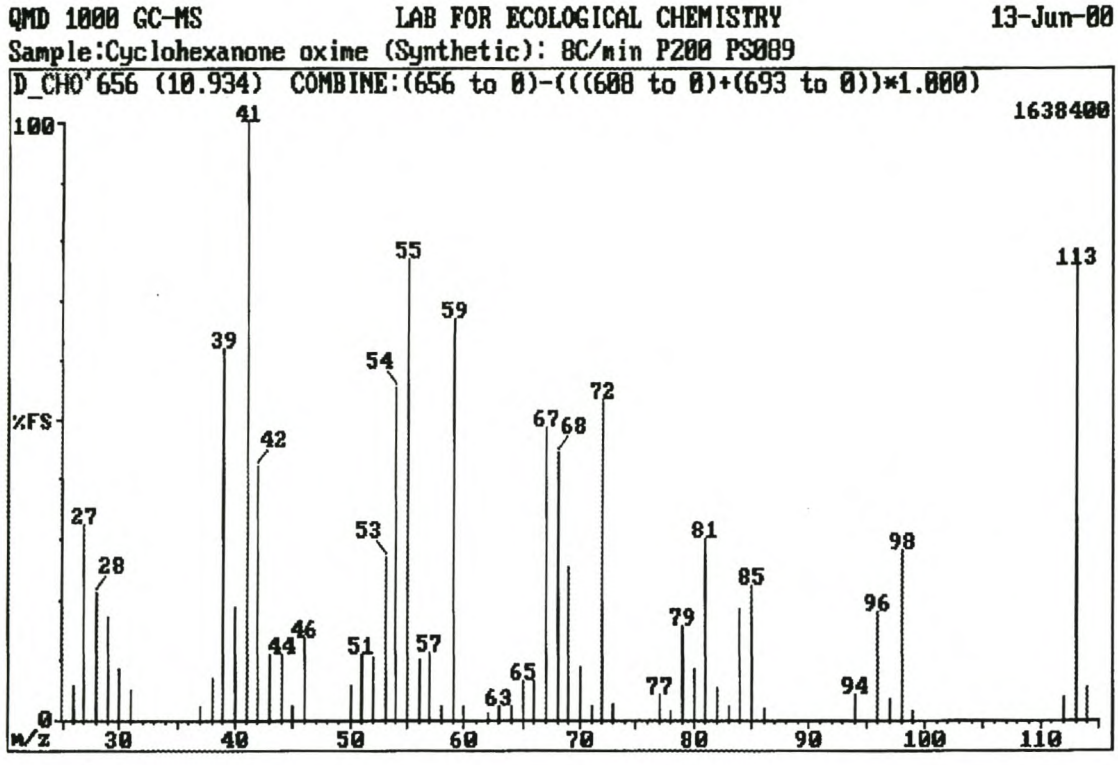

Fig. 3.21 (a): EI mass spectrum of cyclohexanone oxime

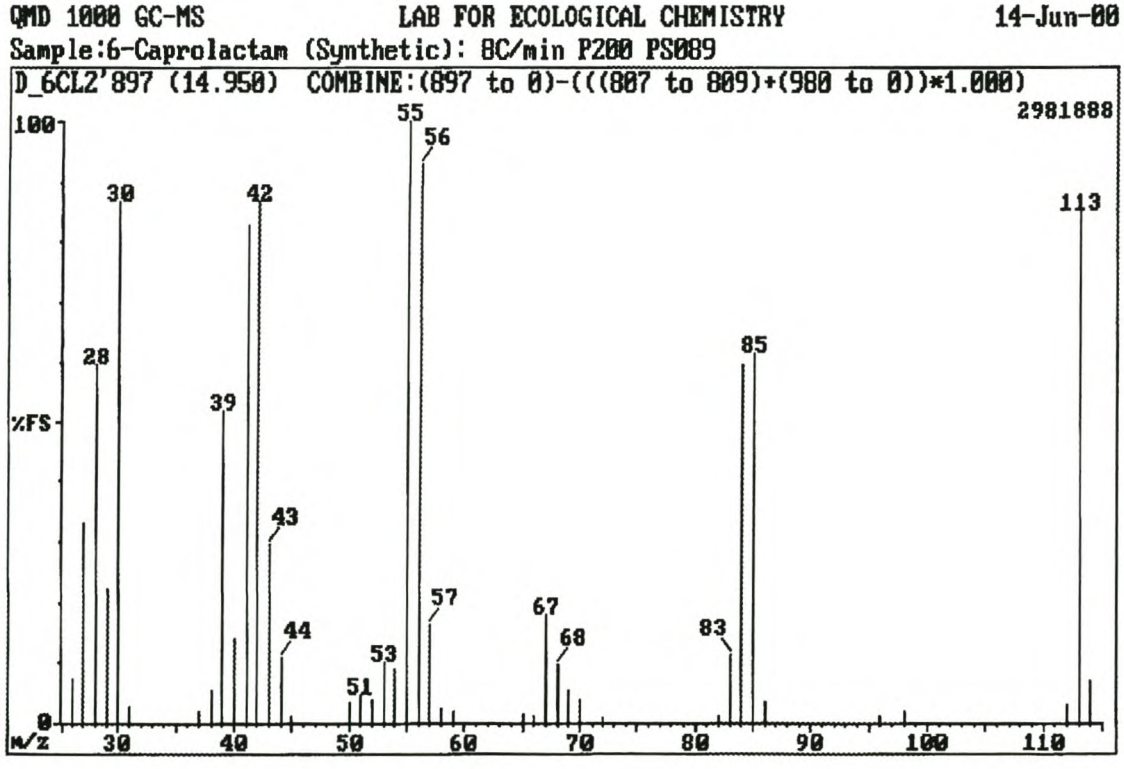

Fig. 3.21 (b): EI mass spectrum of 6-hexanelactam

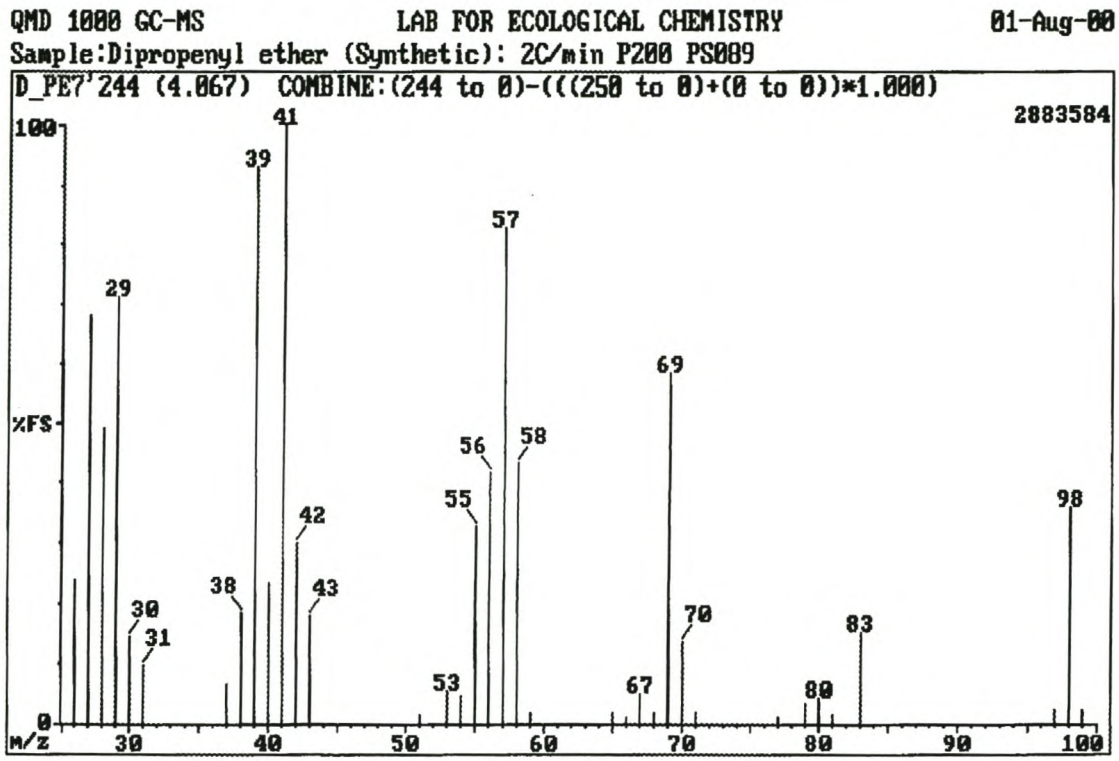

Fig. 3.22 (a): EI mass spectrum of (*Z*,*Z*)-dipropenyl ether

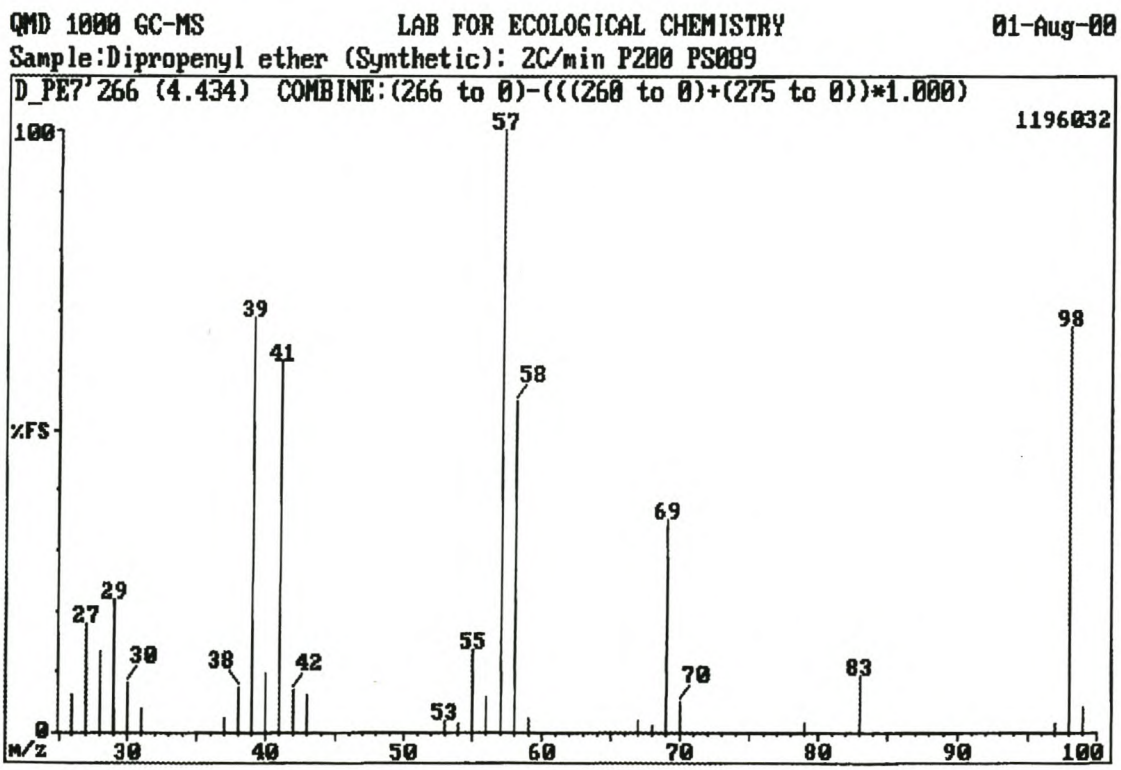

Fig. 3.22 (b): EI mass spectrum of (*E*,*Z*)-dipropenyl ether

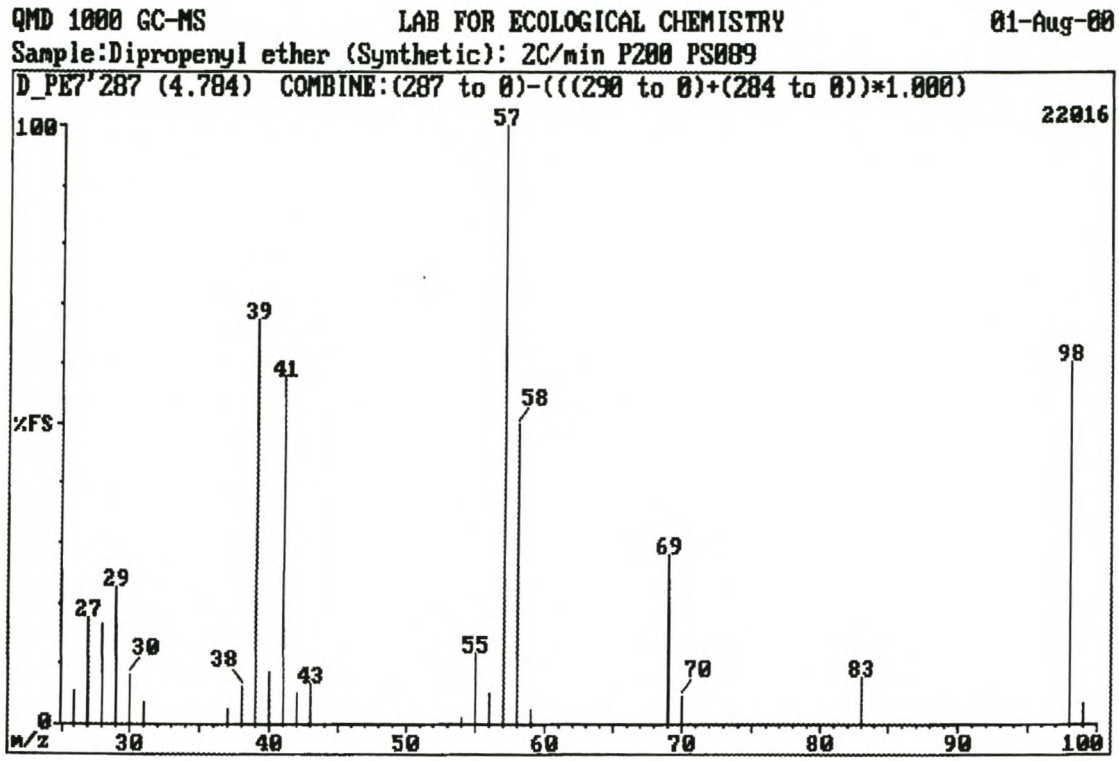

Fig. 3.22 (c): EI mass spectrum of (*E*,*E*)-dipropenyl ether

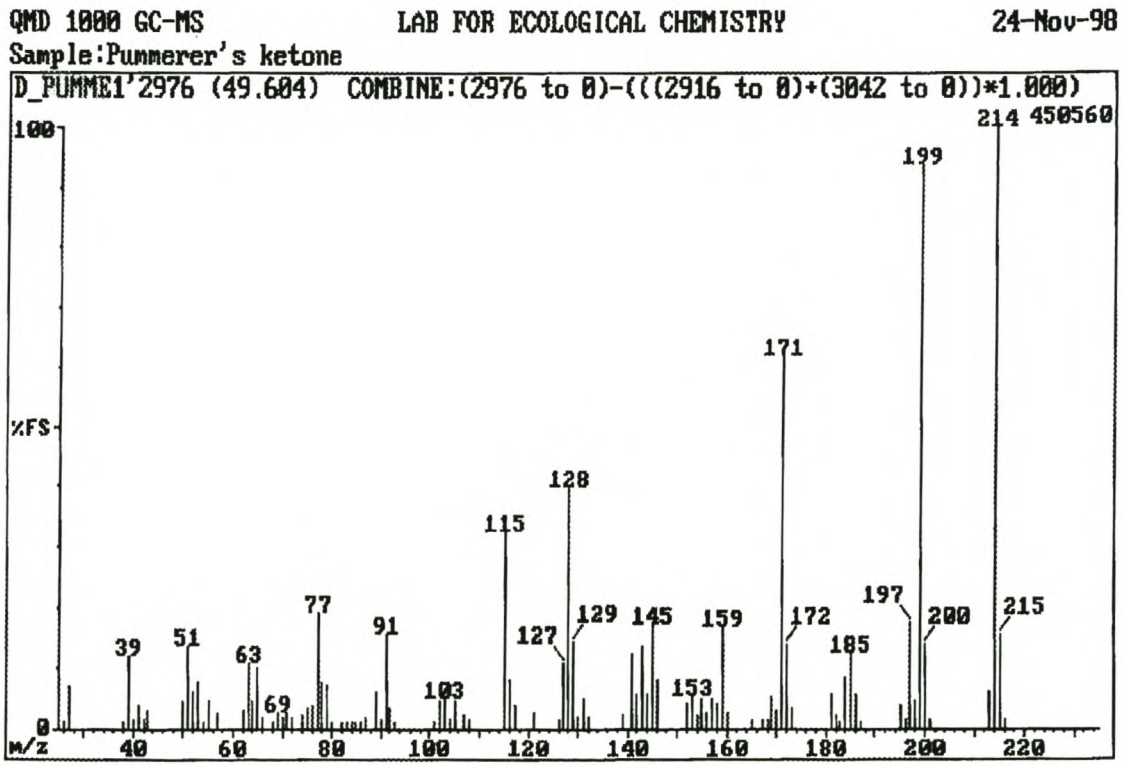

Fig. 3.23: EI mass spectrum of Pummerer's ketone

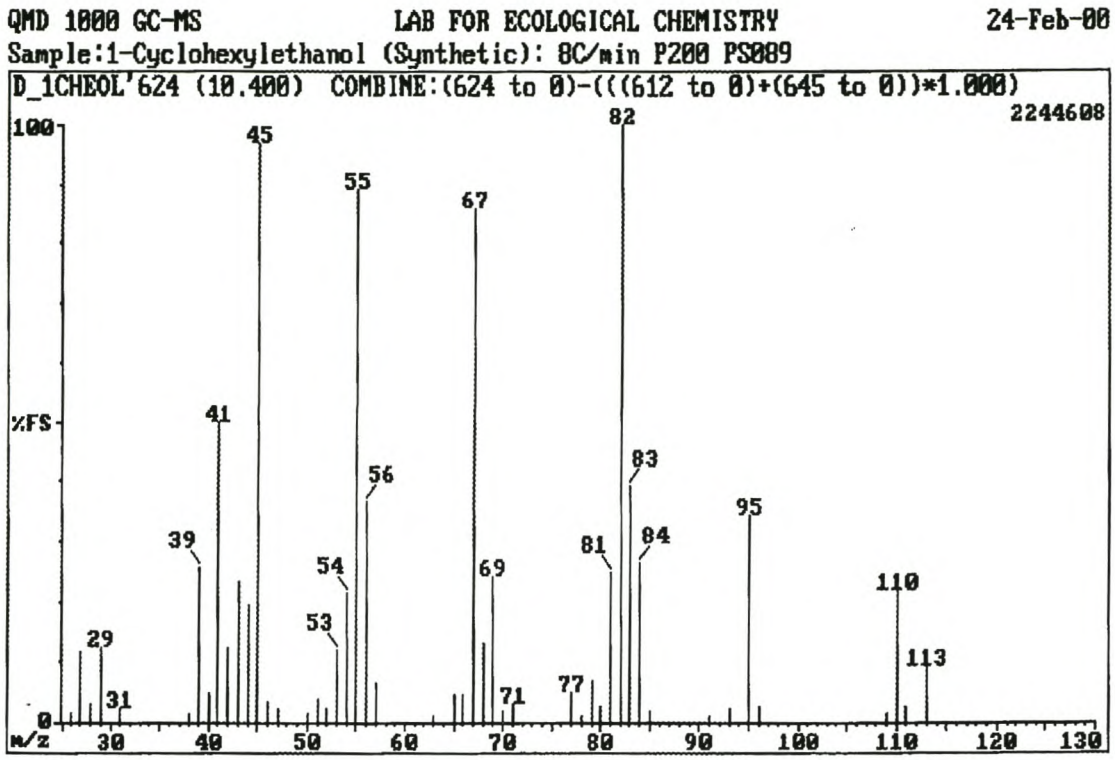

Fig. 3.24 (a): EI mass spectrum of 1-cyclohexylethanol

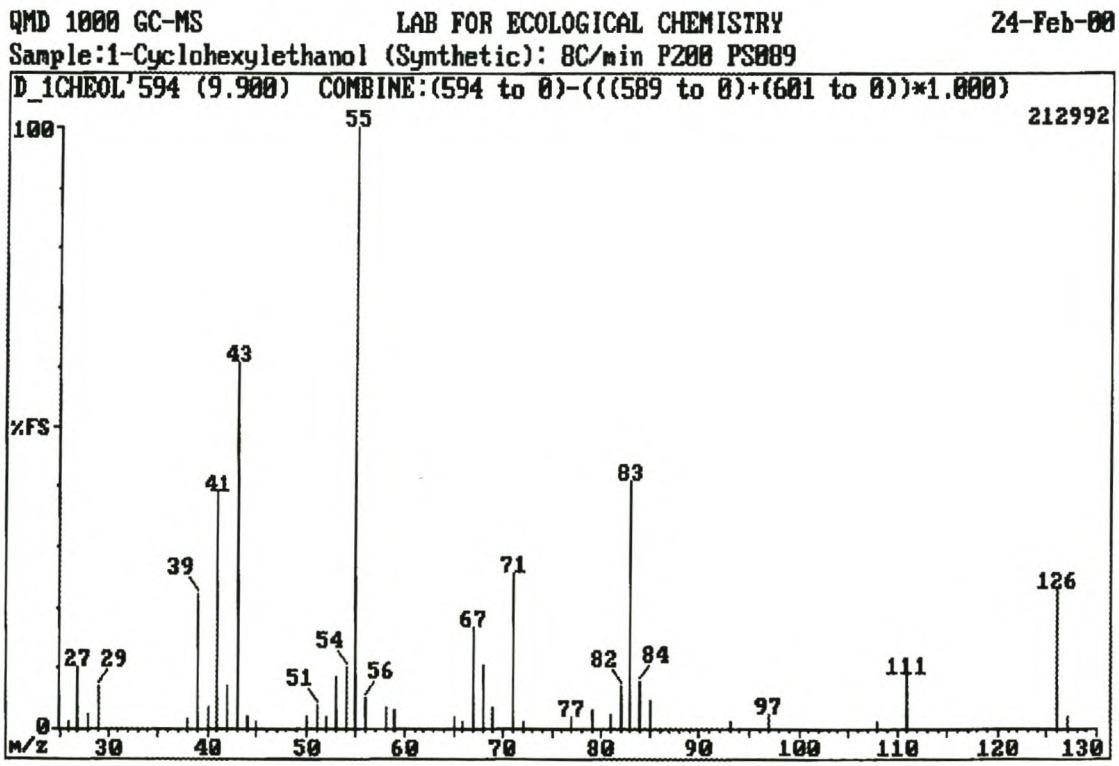

Fig. 3.24 (b): EI mass spectrum of acetylcyclohexane

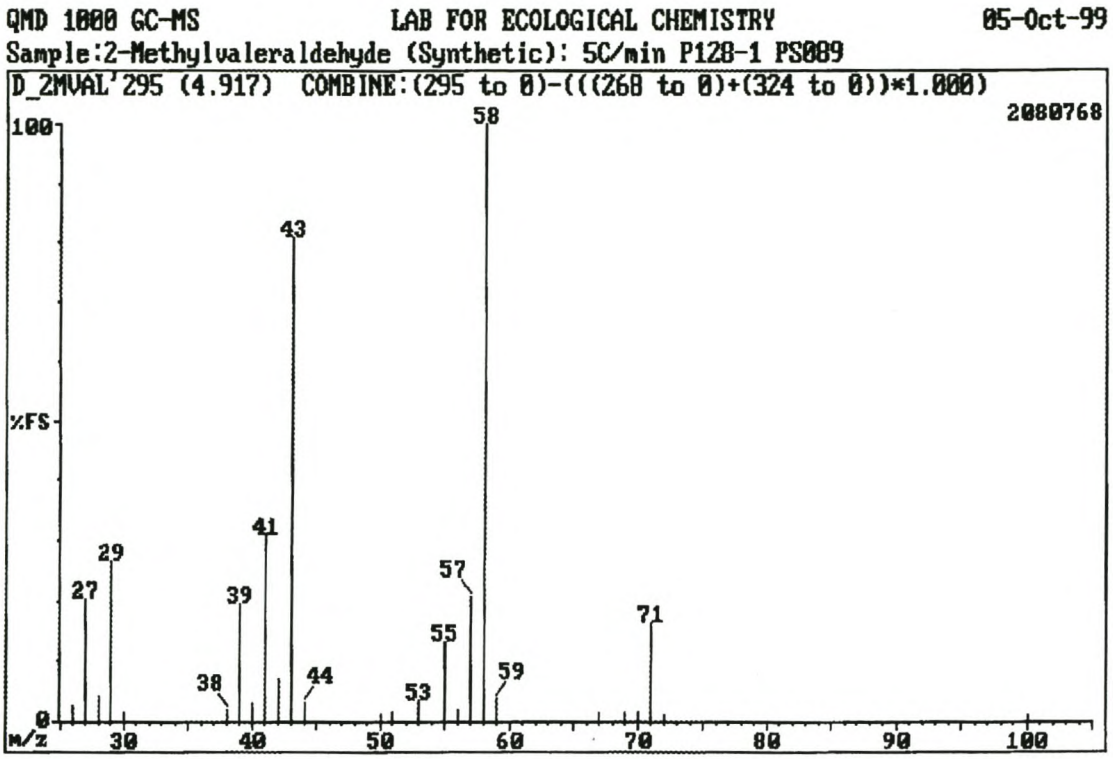

Fig. 3.25 (a): EI mass spectrum of 2-methylpentanal

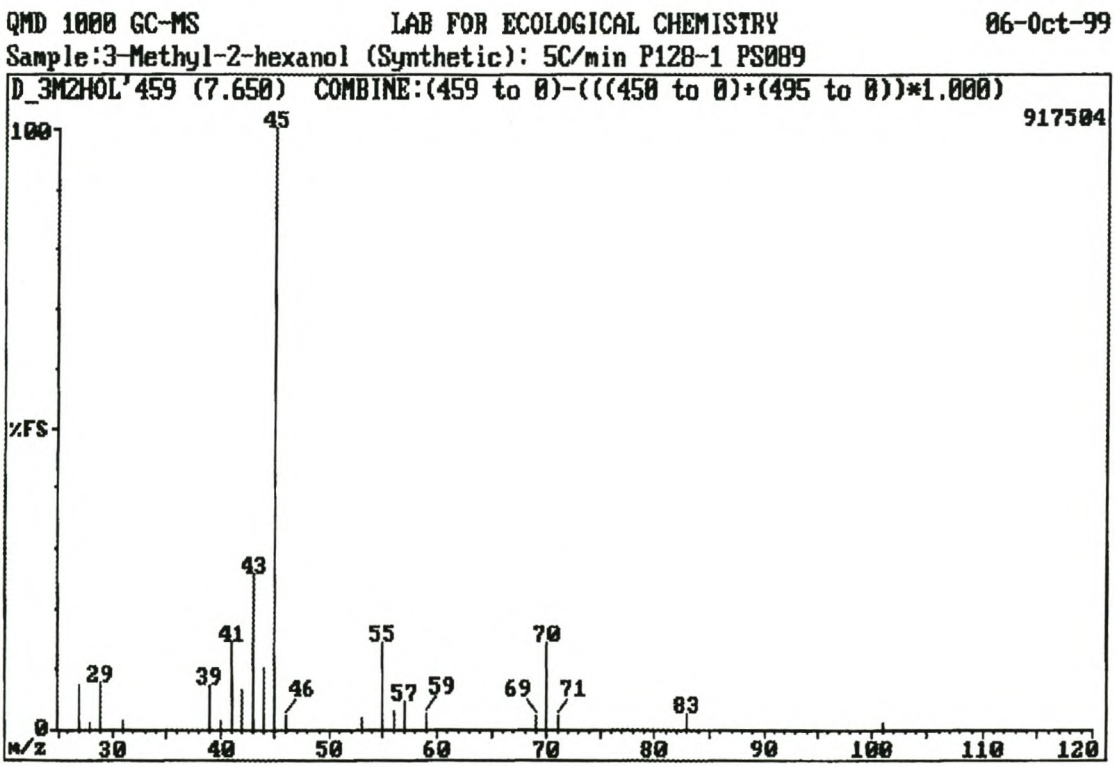

Fig. 3.25 (b): EI mass spectrum of 3-methyl-2-hexanol

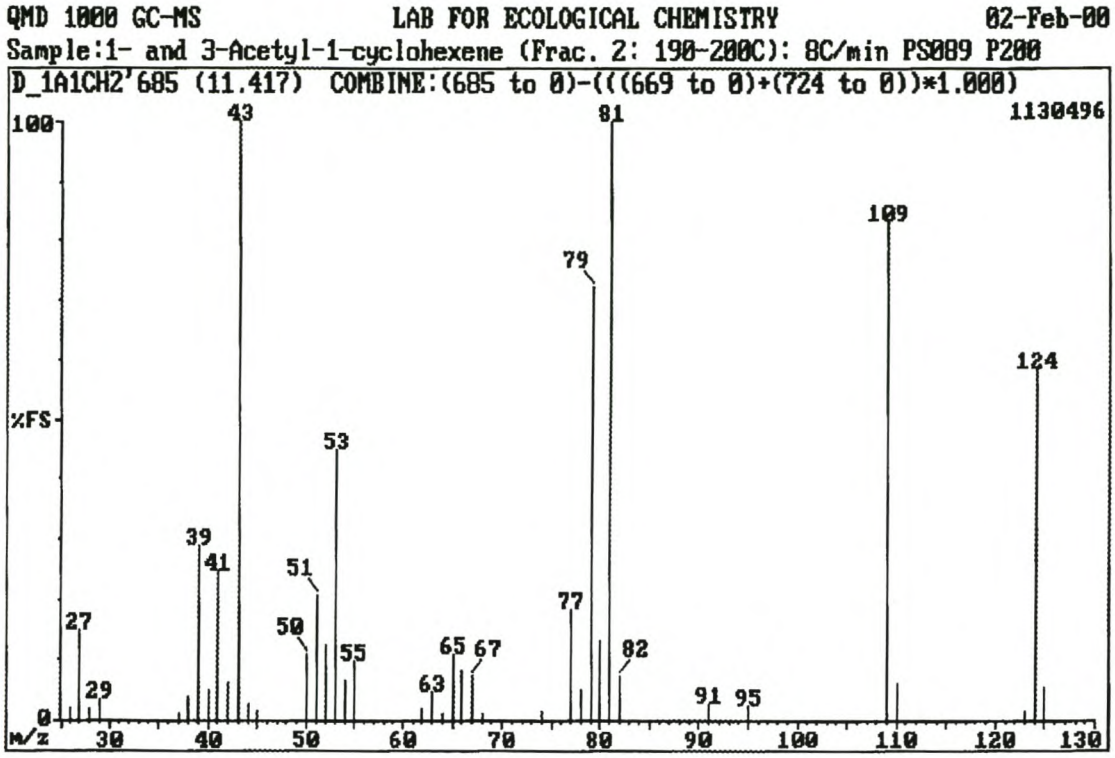

Fig. 3.26 (a): EI mass spectrum of 1-acetyl-1-cyclohexene

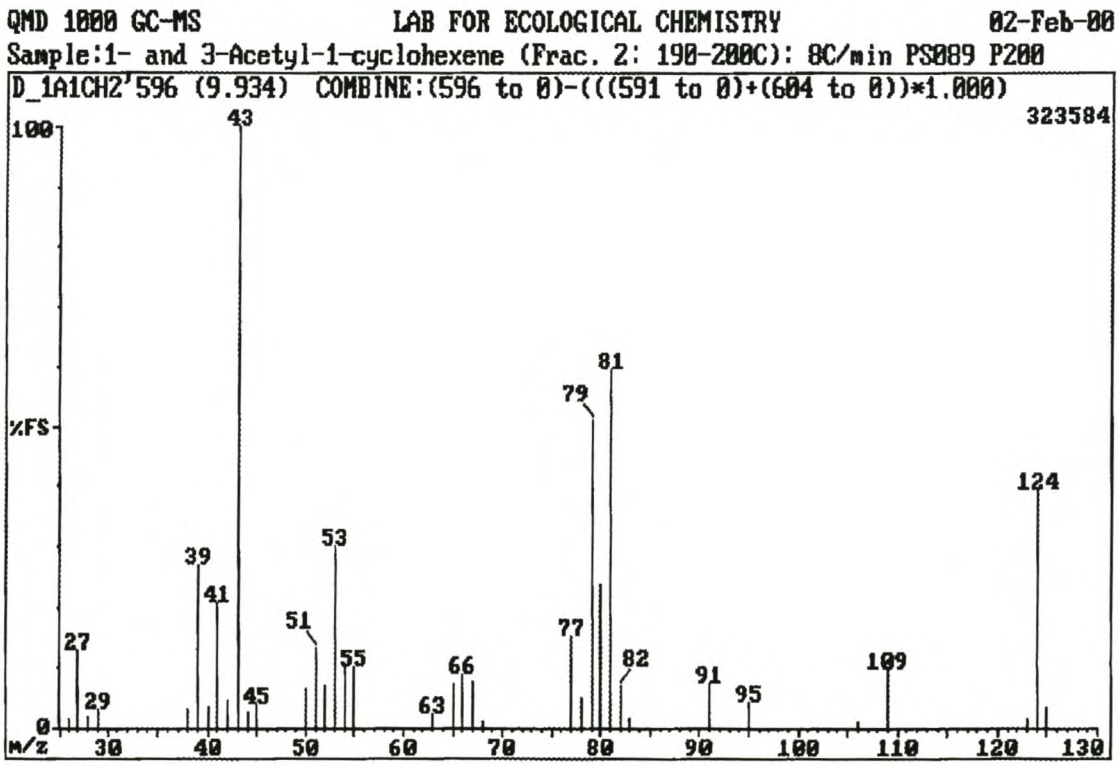

Fig. 3.26 (b): EI mass spectrum of 3-acetyl-1-cyclohexene

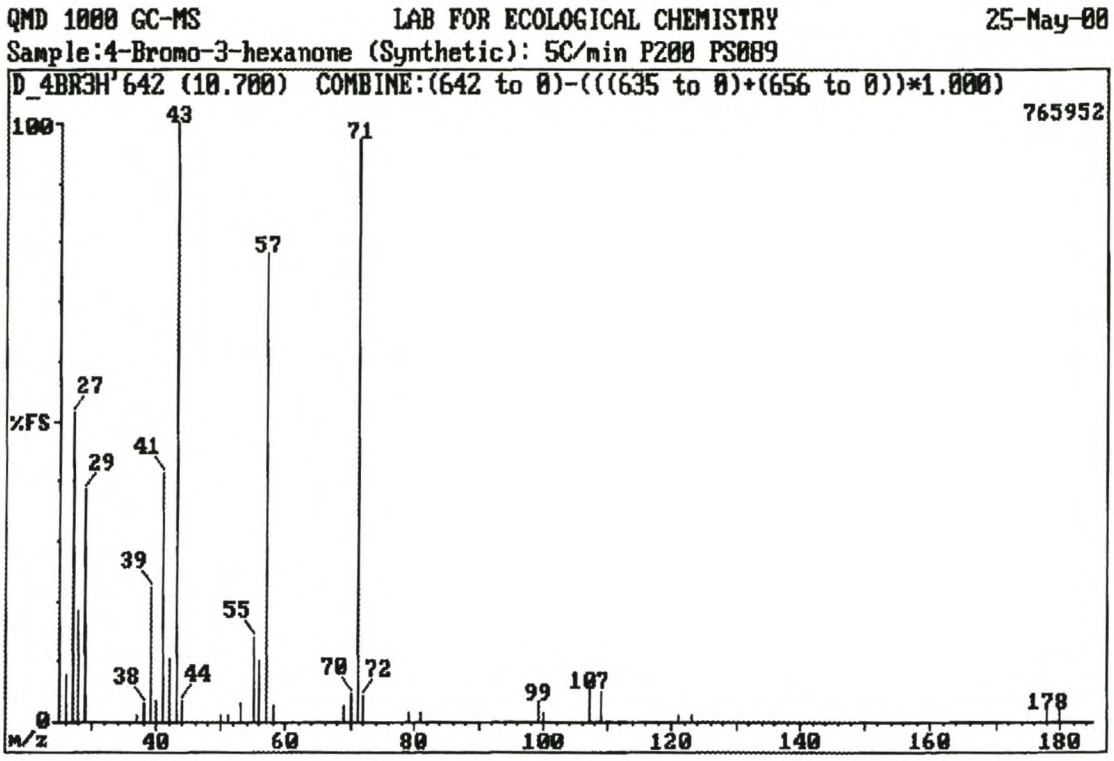

Fig. 3.27 (a) EI mass spectrum of 4-bromo-3-hexanone

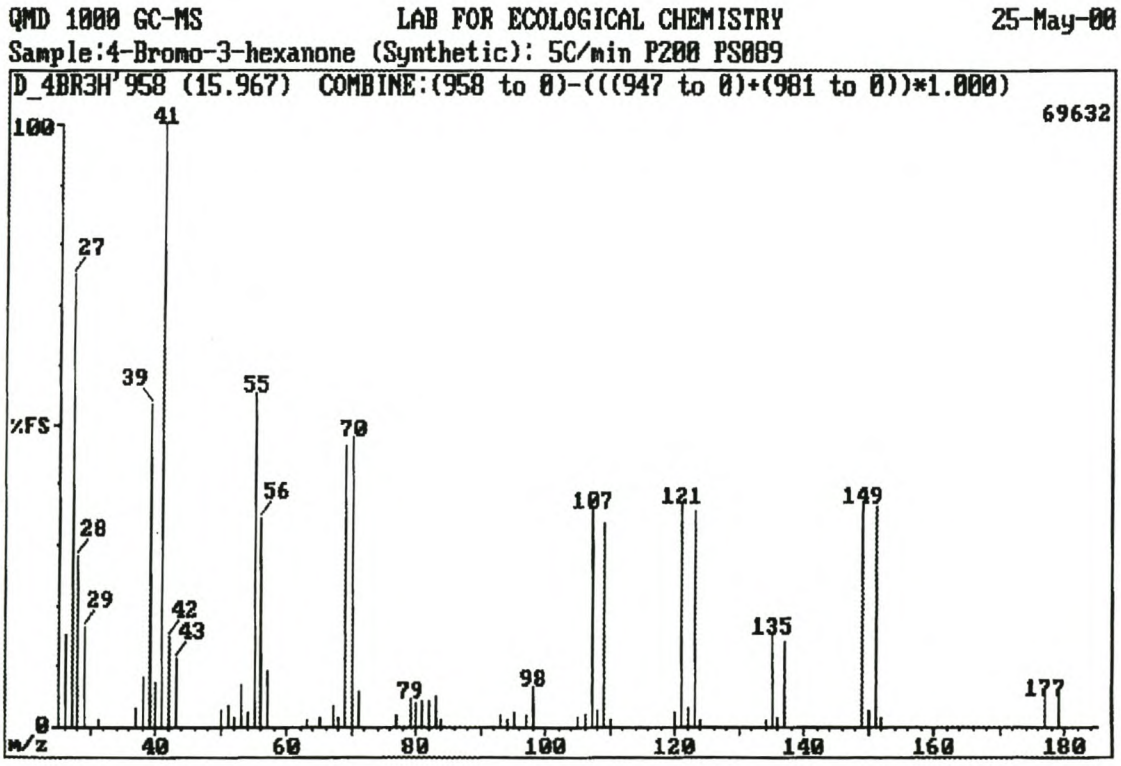

Fig. 3.27 (b) EI mass spectrum of 2-bromo-3-hexanone

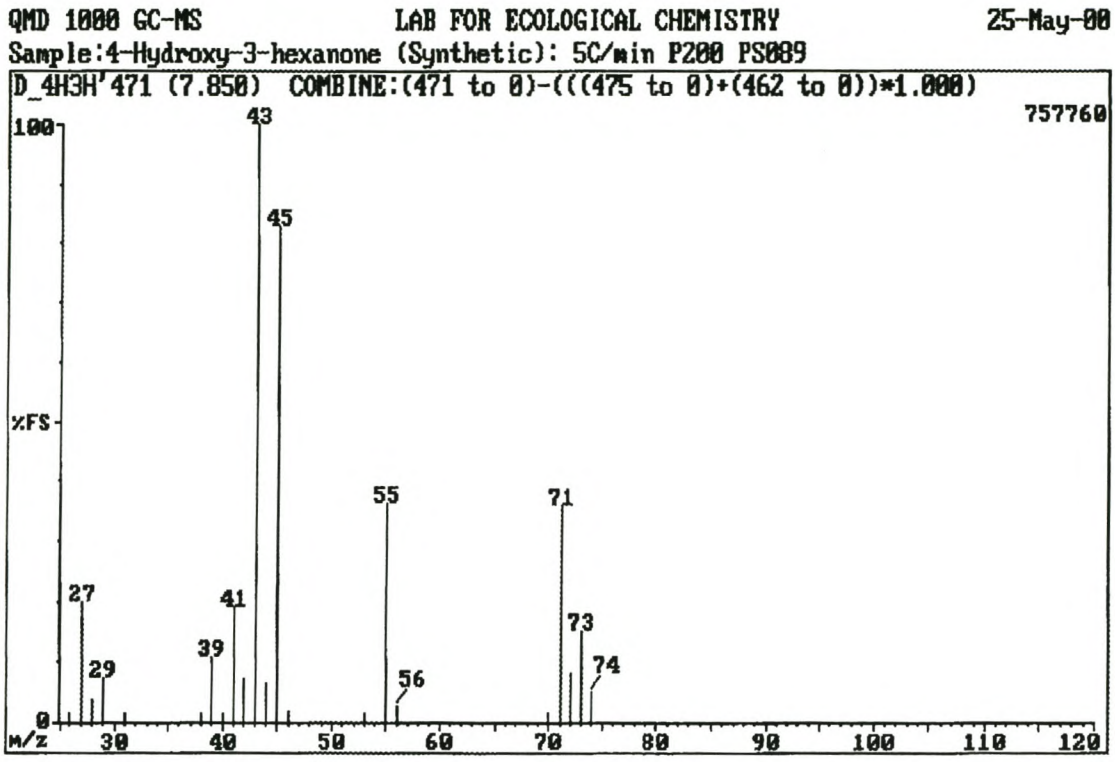

Fig. 3.27 (c) EI mass spectrum of 2-hydroxy-3-hexanone

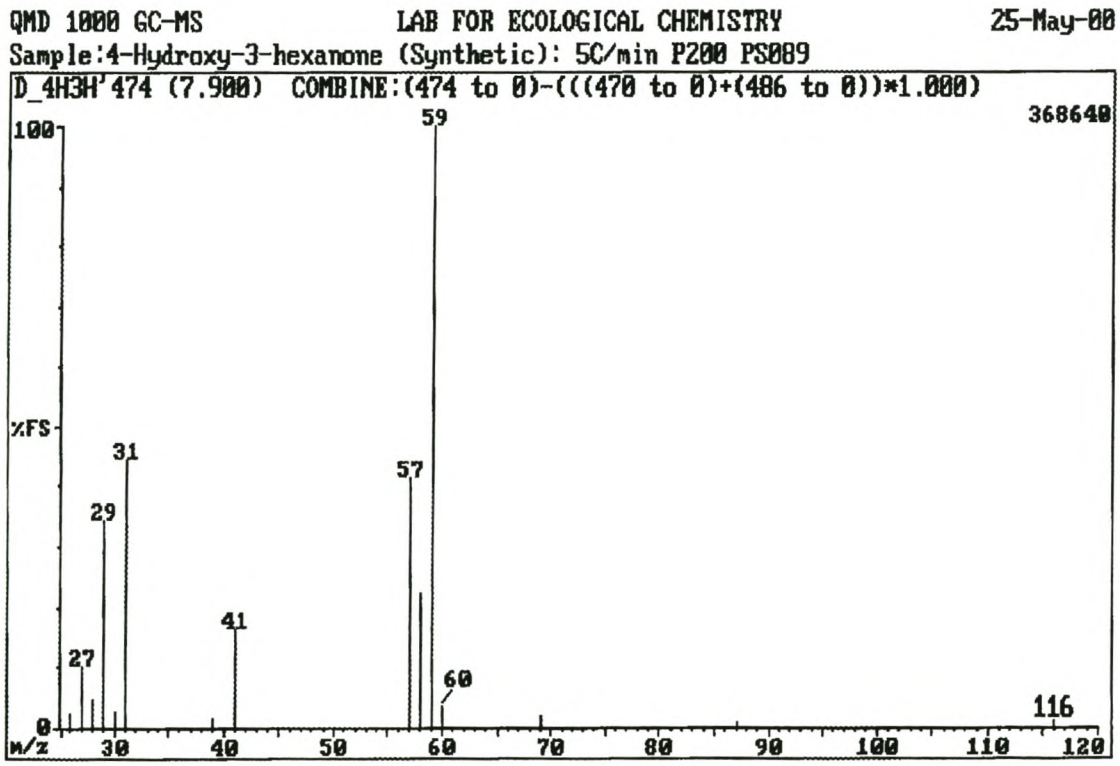

Fig. 3.27 (d) EI mass spectrum of 4-hydroxy-3-hexanone

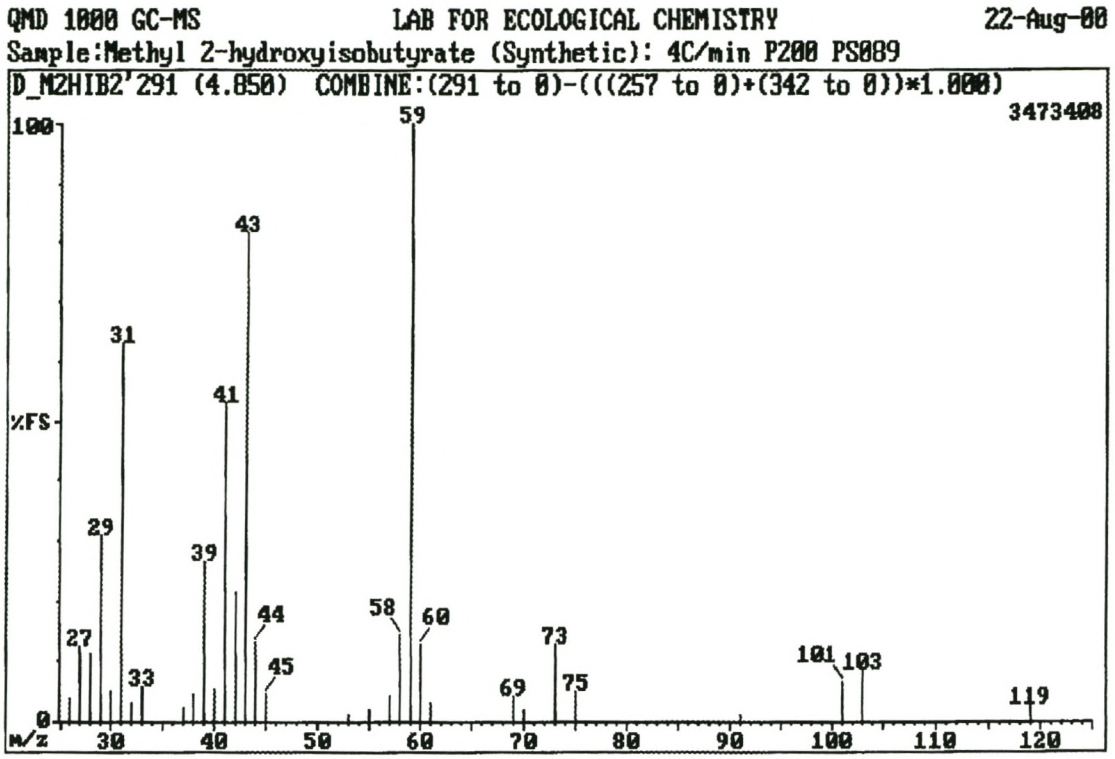

Fig. 3.28: EI mass spectrum of methyl 2-hydroxy-2-methylpropanoate

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